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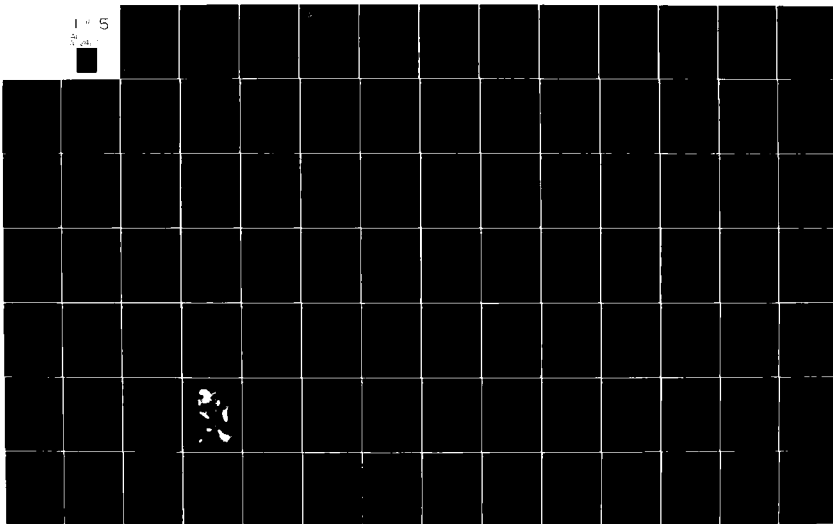
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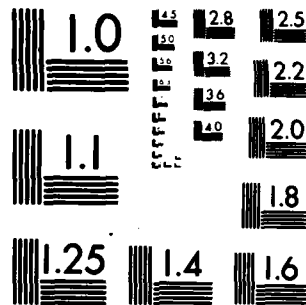
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ANNUAL RESEARCH PROGRESS REPORT

U.S. ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

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DEPARTMENT OF THE ARMY
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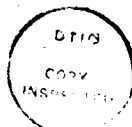
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Basil A. Pruitt Jr.
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COMMANDER & DIRECTOR

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FOREWORD

The burn patient experiences the same pathophysiologic changes as any injured man, with the extent and duration of change in direct proportion to burn size or severity of injury. These changes involve not only the skin, the injury of which is obvious to all, but every other organ system as well. All the early and late disturbances of organ and cell function and complications which occur in burn patients, occur in patients who have sustained mechanical trauma and even those undergoing elective surgery. Consequently, the studies reported herein have direct application to all injured soldiers.

The treatment advances resulting from these and prior studies have not only improved survival of the severely injured, but have both improved and simplified treatment of patients with lesser injuries. In short, the challenge of resuscitation and wound care of patients with relatively minor injuries has been met and no longer serves as a patient care limitation. The formerly common complications of renal failure, gas gangrene and burn wound sepsis have been brought under control and more recent complications, such as acute pulmonary insufficiency have been significantly reduced and ameliorated as a result of the work of the staff of this Institute and other clinically based research organizations. The clinical activities of such research groups serve as both anchor to insure investigative relevance and compass to point the way to further progress.

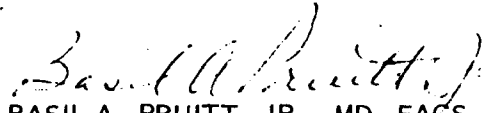

BASIL A. PRUITT, JR., MD, FACS
Colonel, MC
Commander and Director

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<p>23. (U) The Clinical Division of the US Army Institute of Surgical Research continues its role as a major specialized clinical treatment center for thermally injured military personnel. Its main objectives are the investigation and modification of new diagnostic and therapeutic methods for optimum care of the burn patient as well as dissemination of the scientific advances to military and civilian medical treatment centers.</p> <p>24. (U) Thermally injured patients both from the Continental United States and throughout the world are evacuated to the US Army Institute of Surgical Research for intensive inpatient therapy. Carefully controlled evaluation of the efficacy of many treatment modalities is undertaken.</p> <p>25. (U) 7710 - 7809 Two hundred thirty eight seriously burned patients were admitted and treated during 1977. Studies during the resuscitation period have emphasized minimization of fluid volume loading and the role of colloid-containing fluids in resuscitation is being reevaluated. Combination of 133 Xenon lung scan, fiberoptic bronchoscopy and pulmonary function tests permit the accurate diagnosis of inhalation injury. The prophylactic use of steroid therapy has been found ineffective in preventing the sequelae of inhalation injury. Improved nutritional regimens have ameliorated weight loss and body mass erosion in burn patients and have remained effective nonoperative treatment of the superior mesenteric artery syndrome. Both small bowel and colon complications of burn injury have been identified and described. The indications for burn wound excision have been refined and related to both depth and extent of burn with specific criteria established for both scalpel excision at the level of the investing fascia and tangential excision of burned hands.</p>									

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ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED
SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 January 1977 - 31 December 1977

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ABSTRACT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED
SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 January - 31 December 1977

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Two hundred and thirty eight patients were admitted to the Clinical Division of the United States Army Institute of Surgical Research during the calendar year of 1977. The principal emphasis of the Clinical Division has continued to be the provision of optimal care for thermal, chemical and electrically injured patients. In addition, original scientific investigation has continued to form the basis for recommendations for improvement and advances in the care of these patients. Major areas of such clinical research included investigation of hemodynamic alterations, pulmonary responses following injury, cardiac dynamics, infection control and continued studies in the areas of post injury nutrition and metabolism. This report summarizes the activities of the Clinical Division of the U.S. Army Institute of Surgical Research during the calendar year 1977 and catalogues the types of treatment, responses to treatment and complications which have contributed to morbidity and mortality of burn patients.

CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

The Clinical Division of the United States Army Institute of Surgical Research continued throughout the calendar year 1977 to provide clinical care for the thermally injured soldier and other authorized patients.

Two hundred and thirty eight patients were admitted during the period of this report. There were 93 aeromedical evacuation flights for 129 patients of which 88 were within the Continental United States for a total of 124 patients. The five OCONUS flights included two to Puerto Rico, one to Panama, one to Mexico and one to Alaska. Fifty-four percent of all admissions were evacuated by the Institute of Surgical Research burn teams.

CLINICAL MANAGEMENT

The overall management of the thermal, chemical and electrically injured patient as practiced by this Institute has been adequately documented in previous Annual Reports and numerous scientific presentations and publications. Only the major changes in management will be discussed in this report.

Hydrotherapy in the Hubbard tank is currently limited to extensively injured patients who, because of the extent of injury or associated injury cannot shower or be given adequate wound care at the bedside. The routine application of silver sulfadiazine as the mainstay of topical chemotherapy has been modified and is no longer the exclusive or predominate topical chemotherapeutic agent utilized. Silver sulfadiazine is applied to the burn wound and allowed to remain for a period of 12 hours, the wound is then cleansed and mafenide acetate is applied for the next 12 hour period of coverage. This sequential alternation of topical agents is continued throughout the period of topical chemotherapy unless the results of bacteriologic monitoring of the burn wound or metabolic complications dictate selection of a single agent. The microbiologic advantage of alternate applications of two topical chemotherapeutic agents to control a variety of organisms has been clinically apparent.

Tangential excision of hand burns has been accomplished with increasing frequency. The patients who are offered the benefits of tangential excision to the hand are those patients with deep partial thickness injury who would not be expected to spontaneously close their wound within three weeks of injury. Tangential excision is not performed if there is evidence of inhalation injury or if the thermal necrosis is full thickness and may involve extensor tendons. Early excision of deep thermal injury has contributed to the maintenance of hand function if the above mentioned guidelines are followed.

RESEARCH

The Institute of Surgical Research clinical staff conducted numerous scientific investigations during calendar year 1977 which involved the investigation of myocardial responses to injury, resuscitation and infection; pulmonary responses to inhalation injury and infection; infection surveillance and investigation to control burn wound and systemic infection; prevention and treatment of acute gastrointestinal complications of thermal injury; and, continued investigation to identify basic metabolic responses to injury and infection and the nutritional support requirements of severely burned patients.

EDUCATION

Throughout calendar year 1977, the staff of the Clinical Division of the Institute of Surgical Research conducted extensive educational activities for military and civilian professional and paraprofessional personnel. Eleven resident physicians (7 Army and 4 Air Force) from military graduate training programs, 15 medical students, 4 civilian physicians and 6 reserve and National Guard physicians were attached to the Institute of Surgical Research for education and experience in the care of the thermally injured patient. Thirteen scientific publications appeared in refereed medical journals and 184 scientific presentations were conducted for military and civilian medical audiences. Formal educational rounds are conducted by the Institute staff for the Brooke Army Medical Center General Surgical house officers and staff. Numerous presentations at the Academy of Health Sciences and various military installations throughout the Continental United States were also conducted. In addition weekly professional conferences were conducted for and by Institute personnel.

MORBIDITY AND MORTALITY

Seventy of the 234 patients for whom disposition was made during calendar year 1977 died in the hospital for an overall mortality of 29.9%. Seventy nine percent of those patients dying had autopsies. The average day of death was 19.3 days postburn. The average total body surface injury of the patients who died was 56.9% and the average third degree burn was 29%. Two patients died on the day of injury.

Six patients who died (8.6%) were under 15 years of age with an average burn size of 43.6% of the total body surface and the average postburn day of death was 17.3 days. Three of six pediatric aged deaths had autopsies.

Fifty seven of the 70 patients died of sepsis (80%). *Pseudomonas* was the single most common organism responsible for death, 21 or 20% of the deaths were attributed to this organism. The next most common

organism accounting for death was Klebsiella, accounting for eight deaths or 11.4% mortality. Staphylococcus aureus accounted for three deaths (4%). Severe inhalation injury accounted for 13 deaths (18.5%). Table 7 itemizes the specific causes of death in 1977.

COMPLICATIONS

Infection continues to be the most common complication noted. One hundred and forty five patients had positive bacterial blood cultures including 112 Staphylococcus, 56 Pseudomonas, 34 Klebsiella, 26 Escherichia, 25 Enterobacter cloacae and 10 Serratia species.

Bacterial burn wound sepsis or invasion was histologically and/or culturally identified in 57 patients, Candida in three and Phycomycotic lesions in six. The mortality data has been previously discussed.

Ninety-one patients (39%) had associated injuries. Seventy patients (30%) had inhalation injury, 15 patients had multiple lacerations, 16 patients had fractures of extremities, 10 patients had cranial-cervical trauma and three patients had blast injury.

Gastrointestinal complications included hemorrhage in 25 patients (10.7%). Since the inception of antacid/Cimetidine therapy to maintain the gastric pH above 5 and prevent stress ulceration no patient has required operation for acute hemorrhage or perforation. Four patients had acute diverticulosis and 15 had ischemic colitis. Other upper gastrointestinal complications included two esophageal erosions, necrosis of the stomach in three patients, ischemic enteritis in four and superior mesenteric artery obstruction of the duodenum in one patient. Five patients had evidence of cholecystitis and five had jaundice with cholelithiasis. Two patients had pancreatitis. All patients with cholecystitis or pancreatitis were managed nonoperatively.

Acute renal failure was seen in 42 patients, most commonly as a terminal event following sepsis/hypotension. Two patients underwent hemodialysis. Nineteen patients had acute tubular necrosis, four had chronic pyelonephritis and five had acute pyelonephritis.

One burn patient was pregnant when injured and had a spontaneous abortion during the post burn course.

Cardiac complications during 1977 included nine patients with acute congestive heart failure, three patients with proven acute myocardial infarction, and three additional patients with suspected acute myocardial infarctions. Two patients had acute bacterial endocarditis. Seven patients (3%) had suppurative thrombophlebitis, an incidence which has remained essentially unchanged in recent years.

Pulmonary complications included 44 patients with acute bronchopneumonia, four with hemogenous pneumonia, two with aspiration pneumonia, and 15 patients required closed tube thoracostomy for the

treatment of pneumothorax. Three patients had demonstrated pulmonary emboli. Eighty-two patients required mechanical assistance for ventilation during their hospital course.

Orthopedic complications were primarily associated with the initial injury and 20 fractures were seen in 16 patients. Fourteen patients had amputations which included three bilateral and one unilateral lower extremity amputation, three upper extremity amputations, four hand amputations, and 14 digit amputations. Two patients developed heterotopic bone across a joint and one patient had a septic arthritis.

The majority of endocrine abnormalities were discovered at autopsy. Three patients had adrenal necrosis, six had adrenal hemorrhage, three had thyroiditis, and one had a carcinoma of the thyroid. Two patients had pituitary necrosis and one had pituitary hemorrhage.

Serious central nervous system complications included one patient with a subdural hematoma requiring craniotomy, one open skull fracture, one cervical spine fracture, two quadriplegic patients and seven patients with an acute brain syndrome following injury.

Psychiatric problems included four paranoid schizophrenic patients, and three attempted suicides. Eleven psychiatric consultations were obtained from the Brooke Army Medical Center for acute psychiatric conditions. Chronic alcoholism continued to be a major complication seen in 38 (16%) patients and one developed overt delirium tremens.

Metabolic problems included eight patients with pre-existing diabetes mellitus and one patient with immunoglobulin deficiency. Only two patients were admitted with massive weight loss. Twenty three patients required supplemental insulin to control their blood glucose concentrations.

STATISTICAL RESUME

During calendar year 1977 238 thermally injured patients were admitted to the Institute of Surgical Research. There were 234 dispositions during the same period and the subsequent data will be based on those dispositions. There were 195 males and 39 females with an average age of 31.7 years and ranging from 9 months to 85 years of age. The average burn size was 34.7% of the total body surface and 13.9% average full thickness injury. There were 33 patients (14.1%) under 15 years of age with an average age of 4.6 years. The average burn size of the pediatric age group was 25.6% with an average full thickness injury of 9% of the total body surface.

The average hospital stay in 1977 was 41 days, however when convalescent leave for active duty military is excluded, the average hospital

stay was 37 days. The average post burn day of admission to the Institute of Surgical Research was two days. However 40% (94 patients) were admitted on the day of burn. This reflects a continued trend towards earlier admission as compared with 1970 where the average day of admission was 11.2 days.

During the year 1977 1,053 operative procedures were performed on 188 patients or approximately 4.5 operations per patient. Three hundred and thirty five anesthetics were administered to 118 patients for an average of 2.8 anesthetics per patient. Seven hundred and ten ward procedures on 167 patients were performed. Two hundred and fifty six autograft procedures were performed on 108 patients for an average of 2.5 autografting procedures required to cover each patient. Forty three patients had 152 allografting procedures and cadaver allograft was harvested from 65 donors. Porcine xenograft was applied 45 times to 20 patients for an average of approximately 2.5 times per patient. Escharotomies were required in 35 patients (15%) and 14 major amputations were performed in 10 patients principally for treatment of severe electric injury. Tracheostomy was performed in 22 patients or 9.5% of all dispositions and the incidence has remained basically unchanged for several years. One hundred and 45 patients (62% of all dispositions) had at least one positive blood culture drawn during their post burn course. Additional data on infection and burn wound biopsy can be found in the 'Complications' section.

Sixty-two per cent of dispositions (145 patients) received a total of 465,165 cc of blood for an average of 3208 cc per patient. Seven patients had transfusion reactions.

Table 1 identifies the source of admissions of patients during the calendar year 1977. The majority of patients were from Continental United States. Twenty eight patients or approximately 12% were OCONUS. Table 2 summarizes the burn etiology in 1977. The highest mortality group (52.6%) was from structural fires and primarily was related to associated inhalation injury. Gasoline and explosive volatile gases (natural gases, butane, and propane) were responsible for about 35% of the admissions to the Institute of Surgical Research. Table 3 summarizes the effect of age and total body surface injury on mortality and again as expected, the mortality increased with age and extent of burn. One patient in the 80-90% total body surface burn category survived during this period. Table 4 lists mortality rate associated with increments of 10% total body surface burn involvement for the years 1974 through 1977. The overall mortality is essentially the same as in 1976 although the trend is an overall improvement in mortality since 1974 as seen in this chart. Table 6 identifies a statistically significant decrease in mortality in those burns from 30 to 60% as compared with the pre topical chemotherapeutic era of 1962-63 with there being no statistically significant effect evident in those patients with burns that exceed 60% of the total

Table 1. Source of Admission, 1977

Area	A	AD	AF	AFD	N	ND	VAB	Other	TOTAL
1st Army	2	1	0	0	0	0	2	3	8
3rd Army	5	2	2	1	3	1	6	7	27
5th Army	14	11	12	7	4	0	20	84	152
6th Army	1	1	1	2	2	0	5	6	18
Germany	2	1	0	0	0	0	1	0	4
Puerto Rico	0	0	0	0	1	0	0	1	2
Alaska	0	0	0	1	0	0	0	1	2
Canary Islands	0	0	0	0	0	0	0	12	12
Virgin Islands	0	0	0	0	1	0	0	0	1
Hawaii	0	0	1	0	0	0	0	0	1
Japan	0	0	0	0	0	2	0	0	2
Panama	0	0	0	0	0	0	0	1	1
Mexico	0	0	0	0	0	0	0	1	1
Azores	0	0	0	0	1	1	0	0	2
M.D.W.	0	1	0	0	0	0	0	0	1
	24	17	16	11	12	4	34	116	234

A - Army
 AF - Air Force
 D - Dependent
 Other: Civilian Emergency
 US Public Health Service Beneficiary
 Bureau of Employees Compensation Beneficiary
 N - Navy, Marine Corps & US Coast Guard
 VAB - Veterans Administration Beneficiary

Table 2. Burn Etiology, 1977 - 234 Dispositions

Causes	Number of Patients	% Disposition	Deaths	% Mortality
Gasoline & Kerosene	50	21.4%	14	28.0%
Structural Fires	19	8.1%	10	52.6%
Motor Vehicle Accidents	13	5.6%	4	30.8%
Aircraft Accidents	20	8.5%	8	40.0%
Open Flames	21	8.9%	4	19.0%
Electrical	12	5.1%	1	8.3%
Hot Liquid	24	10.3%	6	25.0%
Chemical	3	1.3%	1	33.3%
Others	21	8.9%	8	38.1%
Butane, Propane or Natural Gas Exp.	30	12.8%	11	36.7%
Smoking Clothes Ignited	6	2.6%	2	33.3%
Bomb, Shell, Simulator Grenade, Gunpowder Exp.	11	4.7%	1	9.1%
Tile Cement, Glue	2	0.0%	0	0.0%
Contact	2	0.0	0	0.0%
TOTAL	234		70	

Table 3. Age, Body Surface Involvement & Mortality, 1977

Age (Yrs)	Per Cent Burn										Total		% Mortality
	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Cases	Deaths	
0-1	1	0	0	0	0	0	0	0	0	0	1	0	0.0
1-2	1	3	2(1)	0	0	0	0	0	0	0	6	1	16.7
2-3	0	1	0	4(1)	1(1)	0	0	0	0	0	6	2	33.3
3-4	0	2	0	2	2(1)	1(1)	0	0	0	0	7	2	28.6
4-5	0	0	1	1	0	0	0	0	0	0	2	0	0.0
5-10	1	2	2	1	0	0	0	0	0	0	6	0	0.0
10-15	2	1	0	1	0	0	1(1)	0	0	0	5	1	20.0
15-20	6	3	1	2	4(1)	1	1(1)	1	1	1(1)	21	3	14.3
20-30	14	12	8	14(1)	8(3)	7(2)	6(4)	2(2)	2(2)	1(1)	74	15	20.3
30-40	5	5	3	5(2)	3	4(4)	4(2)	5(5)	1(1)	2(2)	37	16	43.2
40-50	2	0	4	6	3(1)	3(2)	1(1)	0	2(2)	0	21	6	28.6
50-60	3	4	7(1)	3(2)	0	4(2)	3(3)	3(3)	0	0	27	11	40.7
60-70	1	0	2(1)	3(1)	2(1)	0	0	1(1)	0	0	9	4	44.4
70-80	0	0	2(2)	3(2)	1(1)	0	2(2)	0	0	0	8	7	87.5
80-90	1	2(1)	0	1(1)	0	0	0	0	0	0	4	2	50.0

Total	37	35	32	46	24	20	18	12	6	4	234		
Deaths	0	1	5	10	9	11	14	11	5	4		70	
% Mortality	0	2.9	15.6	21.7	37.5	55	77.8	91.7	83.3	100			29.9

Note: Deaths shown in parentheses.

Table 4. Per Cent Body Surface Involvement and Mortality, 1974 - 1977

% Burn	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Total
(1974)											
No. Burned	26	24	28	32	36	28	19	10	14	9	226
Deaths	0	0	4	10	18	17	17	9	13	9	97
% Mortality	0	0	14.3	31.2	50	60.7	89.5	90	92.9	100	42.9
(1975)											
No. Burned	20	36	29	42	28	28	34	19	13	5	254
Deaths	0	1	4	11	8	14	23	16	12	5	94
% Mortality	0	2.8	13.8	26.2	28.6	50	67.6	84.2	92.3	100	37
(1976)											
No. Burned	28	49	39	30	31	27	19	15	13	9	260
Deaths	0	2	3	4	10	13	12	13	13	9	79
% Mortality	0	4.1	7.7	13.3	32.3	48.1	63.2	86.7	100	100	30.4
(1977)											
No. Burned	37	35	32	46	24	20	18	12	6	4	234
Deaths	0	1	5	10	9	11	14	11	5	4	70
% Mortality	0	2.9	15.6	21.7	37.5	55	77.8	91.7	83.3	100	29.9

Table 5. Per Cent Burn Versus Survival, 1955-1977

Year	Survivors (burns over 30%)			Deaths		
	No. Cases	Average % Burn Total	3 ^a	No. Cases	Average % Burn Total	3 ^a
1955	20	39.5	20.3	21	55.6	38.1
1956	22	41.0	17.3	20	57.8	37.8
1957	19	38.4	24.1	17	57.1	38.8
1958	15	42.3	21.6	23	56.5	35.3
1959	29	43.1	20.6	24	63.1	38.1
1960	17	44.2	20.1	30	57.8	37.3
1961	18	44.2	25.0	31	58.0	39.7
1962	18	42.7	21.4	54	59.1	46.2
1963	28	45.8	19.6	57	69.0	41.0
1964	40	41.8	14.8	37	65.0	42.4
1965	47	43.8	21.0	33	66.0	33.4
1966	68	41.5	14.9	59	59.9	31.3
1967	103	42.7	13.3	51	59.9	32.3
1968	143	44.2	12.6	38	54.6	24.6
1969	113	43.2	11.1	70	58.7	26.4
1970	92	39.4	10.7	70	51.9	32.6
1971	63	41.9	14.0	68	60.8	38.0
1972	62	42.0	17.2	103	56.7	35.9
1973	47	43.7	19.6	113	60.3	36.2
1974	55	43.9	12.2	97	60.8	35.9
1975	80	46.1	14.7	94	61.3	32.8
1976	69	45.5	15.0	79	64.2	31.1
1977	66	42.2	14.4	70	56.9	29.0

Table 6. Comparison of Burn Mortality Rates, 1962-1963 and 1964-1977

Per Cent Burn															
Years	0-30			30-40			40-50			50-60			60-100		
	No.	No.	%	No.	No.	%	No.	No.	%	No.	No.	%	No.	No.	%
	Pts. Deaths Mortality			Pts. Deaths Mortality			Pts. Deaths Mortality			Pts. Deaths Mortality			Pts. Deaths Mortality		
1962-63	140	6	4.3	36	16	44.4	36	22	61.1	23	18	78.3	55	49	89.1
1964-77	1908	61	3.2	581	105	18.0	478	153	32.0	322	161	50.0	590	502	85.0

Table 7. Causes of Death, 1977

Patient	Age	Sex	% Burn Total	3° Death	PBD Death	Cause of Death
1	31	M	100	95	0	*Cardiovascular collapse
2	29	M	93	91	0	*Cardiovascular collapse
3	18	F	93	89	14	*Sepsis, <u>E. coli</u> and <u>Staph.</u> , coagulase pos., 2° to burn wound invasion
4	34	M	90	0	8	*Sepsis, organism unknown, probably 2° to inhalation injury
5	30	M	83.5	49.5	3	Sepsis, <u>E. coli</u> , originating in the lungs 2° to inhalation injury
6	45	M	82.5	72.5	1	Cardiovascular collapse, acidosis, respiratory failure 2° to inhalation injury
7	26	M	82.5	57.5	39	Sepsis, <u>Pseudomonas</u> , origin 2° to burn wound invasive infection
8	23	F	82	56	3	Electrolyte imbalance
9	42	M	82	63.5	1	Acute respiratory failure (hypoxia) 2° to inhalation injury
10	57	M	79	39.5	15	*Sepsis, <u>Pseudomonas</u> and <u>Enterobacter</u> , origin 2° to burn wound invasion
11	38	M	76.5	0	6	Sepsis, <u>Pseudomonas</u> , origin 2° to burn wound invasive infection
12	50	F	76	71	13	Sepsis, <u>Phycomycetes</u> , origin 2° to burn wound invasive infection
13	20	F	76	40.5	6	Sepsis, gram negative bacillus, origin 2° to burn wound invasive infection
14	32	M	75	42	6	Sepsis, <u>Pseudomonas</u> , origin 2° to burn wound invasive infection
15	27	M	73	6	3	Cerebral edema with cerebellar tonsillar and uncal herniation
16	58	F	72	62	22	Sepsis, multiple bacterial and mycotic organisms, origin 2° to burn wound invasive infection
17	34	M	72	57	9	Sepsis, <u>Pseudomonas</u> and <u>Enterobacter</u> , origin 2° to pneumonia due to inhalation injury

* Autopsy not performed

Table 7. Causes of Death, 1977

Patient	Age	Sex	% Burn Total	P80 Death	Cause of Death	
18	31	M	72	22	10	Sepsis, <u>Proteus</u> and <u>Pseudomonas</u> , origin 2° to burn wound invasion
19	37	M	71	59	8	Sepsis, origin 2° to burn wound invasion with <u>E. coli</u> , <u>Sp D Enterococcus</u> , and <u>Phycomycetes</u>
20	69	M	70.5	61.5	3	Acute respiratory insufficiency 2° to inhalation injury
21	41	M	69	63	8	Sepsis, <u>Klebsiella</u> , origin 2° to burn wound invasive infection, <u>Pseudomonas</u> also invading burn wound
22	72	M	68.5	28	'3	Sepsis, <u>Pseudomonas</u> , origin 2° to burn wound invasive infection
23	25	F	68	67	'2	*Sepsis, <u>Enterobacter</u> , origin 2° to burn wound invasive infection
24	18	F	65.5	2	6	Sepsis, <u>Pseudomonas</u> , origin 2° to burn wound invasive infection
25	31	M	65	25.5	26	Sepsis, <u>Pseudomonas</u> , origin 2° to burn wound invasive infection
26	11	M	64.5	54	39	Sepsis, <u>Pseudomonas</u> , origin 2° to burn wound invasive infection
27	25	M	64	33	7	Sepsis, <u>Enterobacter</u> , origin 2° to pneumonia due to inhalation injury
28	32	M	63	31.5	7	Sepsis, <u>Pseudomonas</u> , origin 2° to burn wound invasive infection
29	27	M	62.5	56	65	Sepsis, <u>Klebsiella</u> and/or <u>Staphylococcus</u> , origin undetermined
30	25	F	62.5	47	90	Sepsis, <u>Staphylococcus</u> and <u>Pseudomonas</u> , origin of these bacteria respectively endocarditis and burn wound invasive infection
31	50	F	62	0	17	Sepsis, <u>Pseudomonas</u> , origin 2° to burn wound invasive infection
32	50	M	61.5	44	16	Sepsis, organism and origin undetermined
33	58	F	61	30	3	Acute cardiac failure

* Autopsy not performed

Table 7. Causes of Death, 1977

Patient	Age	Sex	% Burn Total	% ⁰ Death	Cause of Death
34	79	F	60	6	7 Sepsis, <u>Klebsiella</u> , origin 2 ⁰ to burn wound invasive infection
35	54	M	58.5	40.5	7 *Sepsis, <u>Klebsiella</u> and <u>Pseudomonas</u> , origin 2 ⁰ to pneumonia due to inhalation injury
36	41	M	58.5	38.5	32 *Sepsis, <u>Staphylococcus</u> and <u>Pseudomonas</u> , origin 2 ⁰ to burn wound invasive infection
37	38	M	58	0	14 Sepsis, <u>Pseudomonas</u> , origin 2 ⁰ to burn wound invasive infection
38	30	M	55	26	12 Sepsis, <u>Pseudomonas</u> , origin 2 ⁰ to burn wound invasive infection
39	21	M	54	35	8 Sepsis, <u>Klebsiella</u> , origin 2 ⁰ to burn wound invasive infection
40	3	M	54	34	32 *Sepsis, organism undetermined, origin probably 2 ⁰ to burn wound invasive infection
41	42	F	54	13	17 Sepsis, organism and origin undetermined
42	25	M	53	0	6 Sepsis, <u>Pseudomonas</u> , origin 2 ⁰ to burn wound invasive infection
43	30	F	52	22.5	47 *Sepsis, <u>Pseudomonas</u> , origin 2 ⁰ to burn wound invasive infection
44	57	F	50.5	31.5	14 *Sepsis, <u>Pseudomonas</u> , origin 2 ⁰ to burn wound invasive infection
45	31	M	40	1	52 Acute respiratory insufficiency, probably 2 ⁰ to acute airway obstruction
46	73	M	49.5	21	22 *Sepsis, <u>Klebsiella</u> , origin 2 ⁰ to pneumonia and emphysema
47	27	M	49.5	1	9 Sepsis, organism undetermined, origin 2 ⁰ to pneumonia following inhalation injury
48	45	M	47	4	13 Sepsis, <u>Enterobacter</u> , origin 2 ⁰ to pneumonia following inhalation injury
49	29	M	47	0	6 Sepsis, <u>Enterobacter</u> , origin 2 ⁰ to burn wound invasive infection

* Autopsy not performed

Table 7. Causes of Death, 1977

Patient	Age	Sex	2 Burn Total	3 ^o	P80 Death	Cause of Death
50	3	M	45.5	0	10	Acute respiratory failure 2 ^o to aspiration
51	69	F	43.5	35.5	12	*Sepsis, <u>Pseudomonas</u> , origin probably 2 ^o to burn wound invasive infection
52	19	M	40	22	10	Sepsis, <u>Pseudomonas</u> , origin 2 ^o to pneumonia following inhalation injury
53	29	M	40	12.5	5	Inhalation injury 2 ^o to bronchopneumonia
54	2	M	40	0	6	*Sepsis, organism and source undetermined
55	31	M	29.5	27	11	Sepsis, organism undetermined, origin 2 ^o to burn wound invasive infection
56	26	M	39.5	12	3	Cardiovascular collapse 2 ^o to massive electrical injury with multi-system organ failure and metabolic derangements (acidosis, hyperkalemia)
57	34	M	38	20.5	56	Sepsis, <u>Klebsiella</u> , origin 2 ^o to burn wound invasive infection
58	65	M	38	14.5	47	Sepsis, organism undetermined, origin probably 2 ^o to pneumonia
59	53	M	36.5	16	33	Sepsis, organism undetermined, origin probably 2 ^o to pneumonia
60	59	M	36	13	36	Sepsis, <u>Pseudomonas</u> , origin 2 ^o to burn wound invasive infection
61	72	F	36	2.5	25	Sepsis, organism and source undetermined
62	81	F	34.5	11.5	17	Sepsis, organism and source undetermined
63	29/12	F	34.5	0	7	Sepsis, probably <u>Pseudomonas</u> , origin 2 ^o to burn wound invasive infection and immune deficiency
64	79	M	30.5	0	15	Sepsis, organism undetermined, probably 2 ^o to pneumonia
65	14/12	F	23	18	10	*Sepsis, <u>Phycomycoses</u> , origin 2 ^o to burn wound invasive infection
66	61	M	22	20.5	137	Sepsis, <u>E. coli</u> , origin 2 ^o to bilateral ascending pyelonephritis

* Autopsy not performed

Table 7. Causes of Death, 1977

Patient	Age	Sex	% Burn Total	3 ^o	P80 Death	Cause of Death
67	59	M	22	0	5	Sepsis, <u>Enterobacter</u> , origin undetermined
68	77	M	20	9.5	84	Sepsis, <u>Staphylococcus</u> and terminal <u>Pseudomonas</u> , origin 2 ^o to septic thrombosis of left brachiocephalic and jugular veins
69	76	F	20	0	35	Sepsis, <u>Klebsiella</u> , origin 2 ^o to peritonitis following multiple colon perforations with ischemic colitis
70	84	M	12.5	1	8	Cardiac arrest, cause unknown

body surface.

SUMMARY

A total of 238 patients were admitted to the U.S. Army Institute of Surgical Research and 234 dispositions were made during calendar year 1977. Topical therapy has changed to alternate applications of silver sulfadiazine and mafenide acetate to maximize the advantages and minimize the disadvantages of each chemotherapeutic agent. Infection continued to be the most common cause of mortality in thermally injured patients. Again in the year 1977 no upper gastrointestinal hemorrhage required operation for control. The continued use of antacid/Cimetadine has eliminated significant upper gastrointestinal hemorrhage from Curling's ulcers. Clinical research continues to contribute to the understanding of the hemodynamic metabolic and infectious complications of the severely injured patients and the results of these studies have led to improved care of the burned soldier.

PRESENTATIONS

Peterson HD: Modern Burn Therapy. Physical Therapy Students, Academy of Health Sciences, Fort Sam Houston, TX 3 Jan 77.

McDougal WS: Treatment of Burns. Residents USAF Sch of Aerospace Med, Brooks AFB, TX 4 Jan 77.

Wilmore DW: Total Parenteral Nutrition. Medical Grand Rounds, Univ of TX Health Sci Ctr at San Antonio. 12 Jan 77.

Sasaki TM and Roberts ML: The Emergency Treatment for Burns. Nursing Care of Burns. Explorer Group, Health Career Fields, San Antonio, TX 13 Jan 77

Peterson HD: Burn Symposium. Eastern Maine Medical Center, Bangor ME 15 Jan 77.

Pruitt BA Jr: Invited Address, Ground Breaking Ceremony, Univ of North Carolina Medical School Burn Center, Chapel Hill, NC 15 Jan 77.

Peterson HD: Current Concepts in Burn Care and Research. 10th Symposium of Military Plastic Surgeons, Walter Reed Army Medical Center, Washington, D.C. 17 Jan 77.

Pruitt BA Jr: 1) Changes in Host Resistance in Injured Man. 2) Opportunistic Infection in Trauma Patients. 3) Suppurative Thrombophlebitis. 4) Current Concepts of Burn Care. 5) Post Injury Metabolism and Nutrition. Horizons in Surgery. Postgraduate Course Univ of Colorado, Denver, CO 16-20 Jan 77.

Sasaki TM: Modern Burn Therapy. Medical Aspects of Advanced Warfare Course, USAF Sch of Aerospace Med, Brooks AFB, TX 19 Jan 77.

Treat RC: Burns. Respiratory Therapy Program. Brooke Army Medical Center, Ft Sam Houston, TX 21 Jan 77.

Peterson HD: Grand Rounds Plastic Surgery Service, Wilford Hall USAF Hospital, Lackland AFB, TX 26 Jan 77.

Peterson HD: Management of Burns. S. Texas Chapter American College of Surgeons, Austin, TX 27 Jan 77.

Lescher TJ: Classification of Burns. Intensive Care Nurse Clinician Course students, BAMC, Ft Sam Houston, TX 2 Feb 77.

Sasaki TM: Burn Wound Therapy. Intensive Care Nurse Clinician Course students, BAMC, Ft Sam Houston, TX 3 Feb 77.

Wilmore DW: Nutrition in the Care of Burn Patients. American

Society for Parenteral and Enteral Nutrition. Chicago, IL 4 Feb 77.

Treat RC: Complications of Burns. Intensive Care Nurse Clinician Course students, BAMC, Ft Sam Houston, TX 7 Feb 77.

Ford D: Occupational Therapy in the Treatment of Thermally Injured Patients. I.C.U. Nursing Students. BAMC, Ft Sam Houston, TX 8 Feb 77.

McDougal WS: Hyperalimentation Therapy. Intensive Care Nurse Clinician Course students, BAMC, Ft Sam Houston, TX 9 Feb 77.

Treat RC: Burns. Junior Science Engineering and Humanities Symposium. Univ of TX in Austin, TX 9 Feb 77.

Ford D: Occupational Therapy in the Treatment of the Thermally Injured Patients. Tri-Service Specialists Course, Academy of Health Sciences, Ft Sam Houston, TX 10 Feb 77.

Sirinek KR: Adverse Cardiodynamic Effects of Vasopression not Avoided by Selective Intraarterial Administration. Soc of Univ Surgeons Annual Meeting, Montreal, Canada 11 Feb 77.

Lescher TJ: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Ft Sam Houston, TX 14 Feb 77.

Wilmore DW: The Use of Fat in Intravenous Nutrition. Massachusetts General Hospital, Boston, MA 15 Feb 77.

Pruitt BA Jr: 1) Nutritional Support of the Burn Patient. 2) Management of Inhalation Injuries. 3) Unsolved Problems in Burn Therapy. Burn Care Symposium. Univ of Cincinnati Medical School. Cincinnati, OH 18-19 Feb 77.

Peterson HD: Electrical Injuries. International Society of Burn Injury, San Juan, P.R. 21 Feb 77.

Peterson HD: Air Evacuation to U.S. Burn Centers; and 2) Special Care During Transportation. Petrochemical Complex, Ponce, P.R. 22 Feb 77.

Treat RC: Burn Assessment and Management. Intern Lecture, AMIC Clinic, BAMC, Ft Sam Houston, TX 22 Feb 77.

Pruitt BA Jr: Diagnosis and Treatment of Opportunistic Infections in Burn Patients. Little Rock Academy of Surgeons, Little Rock, AR 23 Feb 77.

Kaplan JZ: Emergency Care of the Burn Patient. Medical Staff of Weslaco; 2) Fire Department of Weslaco; 3) Nurses of Weslaco, TX 1 Mar 77.

Kaplan JZ: Burn Prevention. Four lectures to: High School students, Lions Club, Kiwanis Club and Volunteer Fire Department, Weslaco,

Tx 2 Mar 77.

Kaplan JZ: Burn Prevention. High School and Middle School students, Donna, TX 3 Mar 77.

Wilmore DW: Energy Requirements for Nitrogen Balance. AMA Symposium on Amino Acids. Denver, CO 3 Mar 77.

McDougal WS: Near Isosmotic Intravenous Caloric Infusions in Traumatized, Critically Ill Patients. AMA Symposium on Amino Acids. Denver, CO 3 Mar 77.

Sirinek KR: Augmented GIP Response to Intraduodenal Glucose by Exogenous Gastrin and Cholecystokinin. Central Surgical Society Annual mtg, Buffalo, NY 4 Mar 77.

Peterson HD: Current Concepts in Burn Care. Irwin Army Hospital, Ft Riley, KS 4 Mar 77.

The following presentations were made at the Wichita State Univ Wichita, KS, Continuing Health Education "The U.S. Army Burn Team" on 5-6 Mar 77:

Peterson HD: 1) Early Medical Management of Burn Patients; 2) Prevention and Treatment of Infection in the Burn Patient; 3) Skin Grafts and Wound Coverage for Burn Patients; 4) Inhalation Injuries of Burn Patients; 5) Reconstructive Surgery and Management

Jesse NF and Ford D: 1) Acute Phase of Burn Treatment; 2) Long Term Care Phase of Burn Patients

Peterson J: Nutritional Management

Wilmore DW: Metabolic Problems Following Thermal Injury. Plastic Surgical Research Council. Williamsburg, VA 7 Mar 77.

Pruitt BA Jr: 1) Shock - Early Resuscitation; 2) Burns. Postgraduate Course on Trauma. Johns Hopkins Hospital, Baltimore, MD 9 Mar 77.

Pruitt BA Jr: 1) Prevention and Control of Gastrointestinal Hemorrhage that Complicates Burns; 2) Diagnosis, Treatment, and Significance of Non Bacterial Infection; 3) American College of Surgeons Continuing Education Course on Trauma. Univ of Cincinnati College of Medicine, Cincinnati, Ohio 10 Mar 77

Wilmore DW: Metabolic Research in Burn Patients. American Society of Parenteral Nutrition, Reno, NV 11 Mar 77.

Pruitt BA Jr: 1) Burn Management - Early Care; 2) Burn Wound Management. Trauma and Clinical Conference Division of Plastic Surgery, Univ of California, San Diego; 3) Current Burn Care. San Diego Society of General Surgeons, San Diego, CA 16 Mar 77.

Kaplan JZ: 1) Burn Prevention and 2) Emergency Care of the Burn Patient. First Aid Students, MacArthur High School, San Antonio, TX 17 Mar 77.

Pruitt BA Jr: 1) Burn Resuscitation; 2) Inhalation Injuries. Post-graduate Course Univ. of Kansas Medical School, Kansas City, KS 18 Mar 77.

Wilmore DW: Influence of the Burn Wound on Local and Systemic Responses to Injury. American Surgical Society, Boca Raton, FL 25 Mar 77.

Wilmore DW: Nutrition in Burn Patients. American College of Surgeons mtg, Los Angeles, CA 29 Mar 77.

The following presentations were made at the American Burn Assn Anl Mtg in Anaheim, CA 31 Mar - 2 Apr 77:

Kaplan JZ: Hot Tap Water: A Significant But Unappreciated Hazard
Wilmore DW: Will Increasing Body Temperature Reduce Burn Hyper-metabolism? A Negative Answer
Peterson HD: Evaluation of Healing Time With Hyperbaric Treatments
Sasaki TM: Burn Wound Manipulation Induced Bacteremia
Aulick LH: Influence of The Burn Wound on Peripheral Circulation in Thermally Injured Patients
Herndon DN: Development and Analysis of an Animal Model of the Post Thermal Injury Hypermetabolic Response

McDougal WS: Renal Function. American College of Physicians Renal Symposium. San Antonio, TX 13 Apr 77.

Treat RC: Burns. Foreign Training Officers, Brooks Air Force Base, TX 15 Apr 77.

The following presentations were made at the anl mtg of the Air Force Clinical Surgeons, San Antonio, TX 18 Apr 77:

Wilmore DW: Current Status of Nutrition in Surgical Patients.
Peterson HD: Burn Scar Revision

Pruitt BA Jr: 1) Evaluation and Initial Management of Burn Patients; 2) Wound Care of Burn Patients; 3) Extremity Burns; 4) Metabolic Changes and Nutrition in Severely Burned Patients; 5) Pulmonary Complications of Thermal Injury; 6) Stress Ulcers in Burn Patients; 7) Electrical and Chemical Burns; 8) Recent Advances in Burns. Consultant Tripler Army Medical Center, Honolulu, HI 18-22 Apr 77.

McDougal WS: Pharmacological Difficulties in the Intensive Care Unit. BAMC Pharmacists, Ft Sam Houston, TX 19 Apr 77.

Kaplan JZ: Burn Assessment and Management. BAMC Interns AMIC-ER Ft Sam Houston, TX 19 Apr 77.

Kaplan JZ: Burn Prevention. Administrative Staff BAMC, Ft Sam Houston, TX 28 Apr 77.

Ford DT: Occupational Therapy for The Thermally Injured Patient. Texas Occupational Therapy Association anl mtg, Dallas, TX 29 Apr 77.

Pruitt BA Jr: The Role of the American Burn Association in Care of Trauma Patients. Annual Mtg American Trauma Society, Houston, TX 30 Apr 77.

Pruitt BA Jr: 1) The Importance of Metabolic and Associated Factors in Patient Risk to Surgical Infections; 2) Management of Sepsis in Burn Patients. Second Annual Clarence E. Stafford Memorial Surgical Symposium Los Angeles, CA 1 May 77.

Kaplan JZ: Burn Prevention. Montgomery Elementary School, San Antonio, TX 3 May 77.

Kaplan JZ: Burn Prevention. Coronado Village Elementary School, San Antonio, TX 8 May 77.

Peterson HD: The Burn Patient. Alamo Heights Rotary Club. San Antonio, TX 10 May 77.

Kaplan JZ: Orthopedic Aspects of Burn Care. Texas Orthopedic Ann mtg Houston, TX 14 May 77.

Kaplan JZ: Burn Prevention. Cub Scouts of Ft Sam Houston, TX 16 May 77.

Wilmore DW: The Effects of Fat in Parenteral Diets. International Conference on Parenteral Nutrition, Bermuda 16 May 77.

Pruitt BA Jr: Report of National Representative to International Society for Burn Injuries. Geneva, Switzerland 16 May 77.

Wilmore DW: Metabolic Response to Injury. Surgical Grand Rounds Columbia University, New York 19 May 77.

Kaplan JZ: Burn Prevention. Paramedics, San Antonio, TX 16, 17, 18, 23, 24 May 77.

Kaplan JZ: Treatment of the Burn Patient. Physical Medicine Assn of San Antonio, 25 May 77.

Wilmore DW: Nutritional Assessment in Surgical Patients. Martinez VA Hospital, Martinez, CA 8 Jun 77.

Wilmore DW: 1) The Role of Energy in Nitrogen Balance; 2) Hypocaloric Protein Diets; 3) Nutrition in the Trauma Patient. Postgraduate

Course in Hospital Nutrition. Univ. of California, San Francisco, CA
9-11 Jun 77.

Treat RC: Burn Assessment and Management. BAMC Interns AMIC-ER,
Ft Sam Houston, TX 21 Jun 77.

The following presentations were presented at the seminar "Current
Management of Thermal Injuries" San Antonio, TX 22-23 Jun 77:

Pruitt BA Jr: Escharotomy, Fasiotomy and Electric Injury
Treat RC: Inhalation Injury
McDougal WS: Fluid Therapy after Resuscitation
Pieniadz CJ: Early Postburn Nursing
Sirinek KR: Initial Burn Wound Care
Pruitt BA Jr: Monitoring of the Burn Wound
Peterson HD: Tangential Excision
Lescher TJ: Use of Physiologic Dressings and Synthetic Skin
Glor BA: Nursing Considerations in the Wound
Roberts ML: Care and Grafting Period
Ford DT and Jesse NF: Occupational Therapy and Physical Therapy
Levine BA: Gastrointestinal Complications
Wilmore DW: Metabolic Consequences of Thermal Injury

The following presentations were made at the ISR Thirtieth Anniver-
sary Seminar, San Antonio, TX 24-25 Jun 77:

Pruitt BA Jr: Welcome Address
Mason AD Jr: Resuscitation of the Burn Patient: Colloid Versus
Electrolyte
Dorethy JF: Burn Resuscitation Monitoring by Echocardiography
Lam V: Effect of Burn Injury and Resuscitation on Extravascular
Lung Water
Treat RC: Laboratory and Clinical Studies of Inhalation Injury
Wilmore DW: Substrate Utilization in Burned Man
Aulick LH: Regional Blood Flow Changes Following Thermal Injury
McDougal WS: Influence of Diet on Hepatic Function

Lescher TJ: Treatment of Burns. Officers Basic Course. Academy of
Health Sciences, Ft Sam Houston, TX 12 Jul 77.

Treat RC: Treatment of Burns. Officers Basic Course. Academy of
Health Sciences, Ft Sam Houston, TX 1 Aug 77.

The following presentations were made at the seminar at Northeast
Technical Community College, Norfolk, NE 4-5 Aug 77:

Peterson HD: Early Medical Management of Burn Patients.
Jesse NF: Early Physical Therapy Management of the Burn Patient
Pieniadz CJ: Nursing Care in the Acute Phase
Peterson HD: Nutritional Management of the Burn Patient, Prevention
and Treatment for Infection in the Burn Patient

Ford DT: Occupational Therapy: Splinting Devices Utilized in the Treatment of the Burn Patient
Jesse NF: The Physical Therapy Program for the Burn Patient
Peterson HD: Inhalation Injury of the Burn Patient
Pieniaz CJ: Psychological Aspects of Burn Care
Peterson HD: Reconstructive Surgery and Management for Burn Patients

Pruitt BA Jr: Current Therapy in Burns. 5th Annual Mtg of the American Association of Physicians Assistants San Antonio, TX 9 Aug 77.

Treat RC: Inhalation Injury. Respiratory Therapy students, BAMC, Ft Sam Houston, TX 11 Aug 77.

Treat RC: Burn Assessment and Management. BAMC Interns AMIC-ER, Ft Sam Houston, TX 16 Aug 77.

Wilmore DW: The Priorities of the Healing Wound in the Injury Response. Surgical Grand Rounds, Univ of TX Health Sci Ctr at San Antonio 19 Aug 77.

Wilmore DW: Metabolic Aspects of Alimentation. Endocrinology Conf. BAMC, Ft Sam Houston, TX 24 Aug 77.

Treat RC: Treatment of the Burn Patient. American College of Emergency Physicians mtg San Antonio, TX 12 Sep 77.

Wilmore DW: 1) The Role of Lipid in Nutrition; 2) Nutritional Support of Burn Patients. Symposium on Parenteral Nutrition. Southampton, England 13-14 Sep 77.

Treat RC: Treatment of the Burn Patient. Amer Soc of Safety Engineers. San Antonio, TX 15 Sep 77.

Pruitt BA Jr: 1) Burn Wound Biopsies in the Clinical Monitoring and Surveillance of Burn Wound Care; 2) Clinical Experiences with the Infusion in the Early Stages of Burn Care. Symposium on Burn Injuries. Tubingen West Germany 23-24 Sep 77.

Treat RC: Treatment of Burns. Officers Basic Course. Academy of Health Sciences, Ft Sam Houston, TX 26 Sep 77.

Wilmore DW: The Endocrine Response to Injury. Combined Endocrine Conference, N.I.H., Bethesda, MD 30 Sep 77.

Pruitt BA Jr: 1) Pulmonary Effects of Burn Therapy; 2) Treatment of Burns. Trauma Symposium - National Naval Medical Center, Bethesda, MD 30 Sep - 1 Oct 77.

Wilmore DW: When Do You Feed Injured Patients. Regional Trauma mtg National Naval Medical Center, Bethesda, MD 1 Oct 77.

The following presentations were made at the Airport Managers mtg "Airport Disasters" in San Antonio, TX 5 Oct 77:

Pruitt BA Jr: Mass Casualty Triage and Emergency Care
Treat RC: Basic Overview of the Emergency Patient
Lescher TJ: Immediate Evacuation Following Disaster Injury

Wilmore DW: Nutritional Support--When and How? Pre and Post Operative Care. Post Graduate Course, American College of Surgeons, Dallas, TX 18 Oct 77.

Pruitt BA Jr: Fundamental Responses to Trauma. American College of Surgeons, Post Graduate Course, Dallas, TX 18 Oct 77.

Treat RC: Burns--Assessment and Emergency Management. BAMC Interns AMIC-ER, Ft Sam Houston, TX 25 Oct 77.

Treat RC: Current Concepts in the Treatment of Burns. Medical and Surgical staff Bergstrom AFB, TX 25 Oct 77.

Wilmore DW: Parenteral Nutrition in a Community Hospital. Methodist Hospital, San Antonio, TX 27 Oct 77.

Treat RC: Current Concepts in the Treatment of Burns. Central Texas Military Physicians Assistants, Univ of TX Health Sci Ctr, San Antonio, TX 28 Oct 77.

Pruitt BA Jr: Opportunistic Infections in Burn Patients. Surgical Grand Rounds, Georgetown Univ. Hospital, Washington, D.C. 29 Oct 77.

Pruitt BA Jr: Current Burn Treatment. Second Annual U.S. Army Medical Symposium, Ft Leonard Wood, MO 29 Oct 77.

Pruitt BA Jr: 1) Fluid Volume and Electrolyte Changes in the Early Postburn Period; 2) Metabolic Change and Nutrition of the Seriously Burned Patient; 3) Current Treatment of Severe Burn Injury. 45th Annual Postgraduate Assembly Omaha Mid-West Clinical Society, Omaha, NE 31 Oct 77.

Treat RC: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Ft Sam Houston, TX 1 Nov 77.

Wilmore DW: 1) Energy Sources in Parenteral Nutrition; 2) Nutritional Support Following Burn Injury. Postgraduate Course on Parenteral Nutrition. Massachusetts General Hospital, Boston, MA 7 Nov 77.

Pruitt BA Jr: The Diagnosis and Treatment of Opportunistic Infections in Burn Patients. Hollywood Academy of Medicine, Hollywood, CA 10 Nov 77.

Pruitt BA Jr: 1) Treatment of Sepsis; 2) Pulmonary and Gastro-intestinal Complications; 3) Future Plans for Burn Care. ABA Postgraduate Burn Seminar, Phoenix, AZ 11-12 Nov 77.

Treat RC: Treatment of Burns. Medical and Surgical Staff, Mercy Hospital, Jourdan, TX 15 Nov 77.

Wilmore DW: Carbohydrate Metabolism in Injured and Septic Patients. AMA Symposium on the Nutritional Support of Critically Ill Patients. San Antonio, TX 18 Nov 77.

Lescher TJ: Treatment of the Burn Patient. MAST-EMS Convention, San Antonio, TX. 1 Dec 77.

Wilmore DW: Nutrition in the Hospitalized Patient. Postgraduate seminar, Univ of TX Health Sci Ctr at San Antonio, TX 1 Dec 77.

Wilmore DW: The Injury Response and the Healing Wound. Surgical Grand Rounds, Yale University Medical School, New Haven, CT 3 Dec 77.

Treat RC: Treatment of the Burn Patient. Industrial Corp-EMT/Safety Engineers Semi-annual mtg, Victoria, TX 8 Dec 77.

Treat RC: Modern Burn Care. Sch of Aerospace Med, Brooks AFB, TX 14 Dec 77.

Pruitt BA Jr: 1) Immediate and Emergency Burn Care; 2) Escharotomy Indication and Technique; 3) Pulmonary Thermal Injury; 4) Systemic Antibiotics; 5) Staffing the Burn Unit; 6) Unsolved Problems in the Burn Patient. Postgraduate Course. Colorado Committee on Trauma of American College of Surgeons, Denver, CO 15-17 Dec 77.

Treat RC: Burns. Respiratory Therapy Program. BAMC, Ft Sam Houston, TX 16 Dec 77.

Treat RC: Burn Assessment and Management. BAMC Interns AMIC-ER, Ft Sam Houston, TX 20 Dec 77.

Pruitt BA Jr: Planning A Burn Unit. Staff Conference Penfield Hospital, Colorado Springs, CO 21 Dec 77.

Treat RC: The Burn Patient. Kiwanis Club Monthly Meeting. San Antonio, TX 27 Dec 77.

PUBLICATIONS

Taylor JW, Wilmore DW, Peterson HD, Pruitt BA Jr: Scalp as a Donor Site. *Amer J Surg* 133:218-220, Feb 77.

Pruitt BA Jr: Multidisciplinary Care and Research for Burn Injury: 1977 Presidential Address, American Burn Association Meeting, *J Trauma* 17:263-269, Apr 77.

Long JM III, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Effect of Carbohydrate and Fat Intake on Nitrogen Excretion During Total Intravenous Feeding. *Ann Surg* 185:417-422, Apr 77.

McDougal WS, Wilmore DW, Pruitt BA Jr: Glucose-Dependent Hepatic Membrane Transport in Nonbacteremic and Bacteremic Thermally Injured Patients. *J Surg Res* 22:697-708, Jun 77.

Sims JK: A Modification of Landmarks for Infraclavicular Approach to Brachial Plexus Block. *Anesth Analg* 56:554-555, Jul-Aug 77.

Pruitt BA Jr: Life-Threatening Complications in the Burn Patient. *Critical Surgical Care* 641-655, 1977.

Wilmore DW, McDougal WS, Peterson JP: Newer Products and Formulas for Alimentation. *Am J Clin Nutr* 30: 1498-1505, Sep 77.

McDougal WS, Wilmore DW, Pruitt BA Jr: Effect of Intravenous Near Isosmotic Nutrient Infusions on Nitrogen Balance in Critically Ill Injured Patients. *SG&O* 145:408-414, Sep 77.

Wilmore DW, Aulick LH, Mason AD Jr and Pruitt BA Jr: Influence of the Burn Wound on Local and Systemic Responses to Injury. *Ann Surg* 186:444-458, Oct 77.

Levine BA, Teegarden DK, McLeod CG, Sirinek, KR, Pruitt BA Jr: Cimetidine Prevents Stress-Induced Gastric Erosions. *Surg Forum XXVIII*, 359-361, 1977.

Herndon DN, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Humoral Mediators of Nontemperature-dependent Hypermetabolism in 50% Burned Adult Rats. *Surg Forum XXVIII* 37-39, 1977.

Welch GW, Petroff PA, Lull RJ, Hander EW, McLeod CG, Clayton WH: The Use of Steroids in Inhalation Injury. *SG&O* 145:539-544, Oct 77.

Aulick LH, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Influence of the Burn Wound on Peripheral Circulation in Thermally Injured Patients. *Am J Physiol* 2:H520-H526, Oct 77.

EXHIBITS

The following exhibit was shown during the year 1977:

"OT-PT Aspects of Burn Therapy" at the 44th Annual Meeting of the American Academy of Orthopedic Surgeons, Las Vegas, NV 3-8 Feb 77.

MOTION PICTURES

The following motion picture was shown during the year 1977:

"Energy Metabolism and Energy Support Following Thermal Injury" at the American College of Surgeons, Dallas, TX 19 Oct 77.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED
SOLDIERS -- ANESTHESIOLOGY

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 January 1977 - 31 December 1977

Investigators:

Anton J. Jirka, MD, Lieutenant Colonel, MC
James K. Sims, MD, Major, MC
Barry Zimmerman, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S1611102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED
SOLDIERS -- ANESTHESIOLOGY

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 January 1977 - 31 December 1977

Investigators: Anton J. Jirka, MD, Lieutenant Colonel, MC
James K. Sims, MD, Major, MC
Barry Zimmerman, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

In the period covered in this report, 344 anesthetics were administered to 129 patients, an average of 2.67 anesthetics per patient. The most commonly used anesthetic agent was Ethrane^R (63.37%), followed by ketamine (12.2%), nitrous oxide (10.2%), and halothane (9%). Regional anesthesia was used for slightly more than 4% of anesthetic administrations.

No serious anesthetic related complications have occurred during this period of time.

Anesthesia

ANESTHESIOLOGY

PREOPERATIVE EVALUATION

Most burn patients are several days postinjury when first seen by the anesthesiologist. In the immediate postburn period, the time is used to gain abundant physiologic data from routine monitoring of various parameters: hematologic (hematocrit, electrolytes, liver and renal function tests), pulmonary (arterial blood gases, respiratory rate, daily chest x-rays), cardiovascular (blood pressure, central venous pressure, cardiac index measured by way of Swan-Ganz catheters), and renal (urine output, urine chemistry), in addition to the usual preoperative patient interview and physical examination.

All patients, regardless of age, who have electrical injuries have a preoperative electrocardiogram performed to rule out possible myocardial damage.

PREOPERATIVE PREPARATION

All patients are kept NPO after 2400 the day prior to surgery with the exception of patients with protected airways (intubation by some route plus tube feedings) and children, who may receive clear liquids up to five hours prior to surgery.

PREMEDICATION

Most burn patients require some pain relief during the trip to the operating room, and most receive a narcotic such as morphine sulfate, 0.1 mg/kg, to a maximal dose of 10 mg, one hour prior to surgery. Glycopyrrolate (Robinul^R), 0.005 mg/kg to a maximal dose of 0.4 mg, is used to dry secretions. Both of these medications are delivered intramuscularly.

Valium (PO) plus Robinul in the above dosage, are used as premedication prior to regional anesthesia.

FLUIDS

All fluids are changed to D₅RL on arrival in the operating room.

TYPES OF ANESTHESIA

The pattern of anesthetic administration has changed from previous years and involves a greater use of enflurane and a lesser use of ketamine. The reasons for this change will be discussed under individual agent headings.

TABLE 1. PRIMARY AGENTS - 1977

	<u>No. of Cases</u>	<u>% of Total</u>
Enflurane	219	63.37
Ketamine	42	12.2
Halothane	31	9.01
N ₂ O	35	10.17
Local	14	4.07
Barbituate	4	1.16

1. Enflurane (Ethrane^R)

Enflurane is a halogenated ether which has been commercially available for approximately the past five years. It has a rapid induction with good muscle relaxation. Biotransformation amounts to less than 2% of an inhaled dose, a fact which perhaps accounts for the few clinical toxic effects observed in spite of the fact that increased plasma fluoride ion concentrations have been observed after administration to patients taking hepatic enzyme inducing drugs. Plasma fluoride levels in hypermetabolic burn patients during and after Ethrane administration have not been measured.

2. Halothane^R (Fluothane)

The use of halothane is avoided mostly for less than rational reasons related to descriptions of probable hepatotoxicity (incidence 0.7 per 1000) in the literature. Previous studies at the Institute of Surgical Research show its repeated use to be safe in the thermally injured patient, and the National Halothane Study showed halothane to be the anesthetic with the best overall mortality rate. It is a smooth anesthetic, unsurpassed as an agent for pediatric patients. This anesthetic is mainly used now for asthmatics, patients with digitalis toxicity, and children.

3. Nitrous oxide

This agent must be used in concentrations of 65-70% with oxygen to assure patient lack of recall of the procedure; thus,

it fails to meet the criterion of being able to provide high oxygen concentrations to the hypermetabolic burn patients. Pancuronium is the only relaxant used in conjunction with this agent. Succinylcholine has not been used for any purpose in this unit for more than three years.

4. Ketamine

This agent is used both IM and IV to produce its characteristic dissociative state, with preservation of basal functions (breathing) and laryngeal reflexes plus secondary catechol stimulation of the cardiovascular system.

Unfortunately, ketamine shares with its parent compound, phencyclidine, the production of a high incidence of unpleasant hallucinogenic side effects. There seems to be a "batch" difference in ketamine, and that possessed by ISR in the past has had an almost 100% incidence of these effects. New methods of administering the drug, as well as various methods of premedication and patient preparation, appear to have reduced the unpleasant emergence reactions to less than 15%. Laryngospasm, airway obstruction, and regurgitation can occur with ketamine. Pronounced blepharospasm prevents its use in eye cases.

5. Subanesthetic Ketamine

Subanesthetic ketamine (single dose 1.5-2 mg/kg IM) has seldom been used during this reporting period. Several factors limit the usefulness of this agent. The Hubbard tank restricts the use of intravenous administration at times, and only procedures with low projected blood loss are scheduled for the tank room; repeated use of ketamine may lead to a buildup of tolerance to the drug's beneficial effects. Larger and larger doses must be used, with sequential administration leading to a greater incidence of undesirable side effects, and multiple anesthetic administrations, spaced closely together, interfere with the feeding schedule of patients, resulting in delayed wound healing.

It should be noted that large doses of narcotics are most definitely not used for tank procedures. The pain encountered is not of the type which is relieved by narcotics, and tank procedures are of short duration. Narcotic use provides little pain relief and may lead to postoperative respiratory depression.

Short, repeated painful procedures should be carried out with a minimum of sedation, to the limit of the patient's tolerance, and narcotic premedication should only be used in small doses.

General anesthesia is required for procedures in which pain is not alleviated by minimum narcotic sedation.

6. Regional Anesthesia

Regional anesthesia is generally considered one of the safest methods available, but its use in the thermally injured patient is limited for several reasons: sepsis and infection of the skin over the site of injection are contraindications for use, and multiple-site operations also limit the practicality of this method.

MONITORING TECHNIQUES

A. CIRCULATION

1. Precordial and/or esophageal stethoscope
2. Peripheral pulse
3. Blood pressure, usually by Infrasonde,^R but also may be by Riva Rocci method. Direct arterial lines have been used when necessary.

4. CVP
5. Swan Ganz catheter
6. ECG
7. Sponge weight - rarely used
8. Urine output

B. RESPIRATION

1. Rate
2. Auscultation
3. Arterial blood gases

C. TEMPERATURE

Most cases now have a temperature monitor. Because of the greatly increased evaporative heat losses in burn patients, hypothermia is a serious problem. Several methods are employed to maintain body temperature during anesthesia:

1. Ambient temperature is maintained at 80-85°F. This is probably the most important of all things done to reduce heat loss.
2. The anesthetic gases may be heated and humidified.
3. A circle system may be used to minimize heat loss.
4. Radiant heat lamps.
5. A K-thermia heating blanket can also be used. It is probably used most effectively on children weighing less than 10 kg and in cooling febrile patients.

TABLE 2. OVERALL PATIENT DATA, USAISR (1966-1977)

Year	No. of Patients	No. Patients Anesthetized		Total Anesthetics (ISR Only)	Anesthetics		Average Per Cent Burn
		(ISR Only)	No. Patients Anesthetized (x100)		No. Patients Anesthetized (x100)		
1966	311	181	58.2	713	3.94	30	
1967	389	239	61.4	670	2.80	28	
1968	311	259	66.6	794	3.07	30	
1969	294	189	64.3	601	3.18	36	
1970	321	198	61.7	497	2.51	30	
1971	301	179	59.5	475	2.65	31	
1972	301	183	60.8	575	3.14	34	
1973	273	141	51.6	377	2.67	38.5	
1974	226	123	54.4	380	3.09	41.57	
1975	254	142	55.9	490	3.45	42.1	
1976	277	139	50.2	476	3.43	37	
1977	242	129	53.3	344	2.67	33.57	

TABLE 3. NATURE OF SURGERY, USAISR (PER CENT)

Procedure	1971	1972	1973	1974	1975	1976	1977
Excision	15.5	19.7	21.5	22.60	25.0	23.0	19.7
Autograft	52.9	51.3	52.6	56.9	51.0	53.0	52.6
Orthopedics	13.0	8.9	8.0	8.1	8.0	5.0	6.2
Chondrectomy	4.0	3.1	2.6	1.60	1.0	2.0	1.9
Eye and lid	3.8	0.7	1.8	1.60	2.0	3.0	2.7
Intra-abdominal	1.7	7.8	2.1	3.70	2.0	1	1
Plastic*							5.7
Other	4.4	1.9	4.8	3.70	11.0	13.0	10.9

*Accounting classification began for 1977 annual report

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED
SOLDIERS: A DESCRIPTIVE COMPARATIVE ANALYSIS OF
PERCEIVED STRESSFUL EVENTS, PSYCHOPHYSIOLOGICAL
SYMPTOMS AND DEPRESSED AFFECT AMONG NURSING PERSONNEL

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

Daniel J. Pesut, Captain, ANC, MSN
Lucinda L. Carlson, Captain, ANC, BSN

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED
SOLDIERS: A DESCRIPTIVE COMPARATIVE ANALYSIS OF
PERCEIVED STRESSFUL EVENTS, PSYCHOPHYSIOLOGICAL
SYMPTOMS AND DEPRESSED AFFECT AMONG NURSING PERSONNEL

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

Investigators: Daniel J. Pesut, Captain, ANC, MSN
Lucinda L. Carlson, Captain, ANC, BSN

It has been hypothesized that the occupational stress associated with burn nursing and burn care may have numerous psychosocial and physiological ramifications. This study was designed to analyze the relationship of perceived stressful life events, psychophysiological symptoms, and depressed moods of nursing personnel assigned to a large burn center.

Forty-seven members of the nursing staff, which was a sample of convenience, completed a 91-item paper and pencil questionnaire. Components of the questionnaire were the Social Readjustment Rating Scale (SRRS), a 22-item screening inventory of Psychophysiological Symptoms, and the Center for Epidemiological Studies Depression Scale (CES-D).

Data were analyzed using a computer program of stepwise multiple linear regression which yielded Pearson Product Moment Correlations. The results of this study indicate significant ($p < .05$) correlations between symptoms and depression (.656), symptoms and life change (.334) and depression and life change (.309). Ninety-nine percent of the personnel in this sample were assessed as moderately or highly depressed.

The findings of this study corroborates some of the conclusions of other investigators, in that significant associations between depressed mood and symptom scores are noted. Discussion focuses on the psychological and physiological implications of depressed affect in burn nursing personnel.

It is concluded that further exploration of occupational stress in burn care settings be undertaken and that systematic study might reveal that adaptation may not be the predominate state in which people function.

A DESCRIPTIVE COMPARATIVE ANALYSIS OF PERCEIVED STRESSFUL EVENTS,
PSYCHOPHYSIOLOGICAL SYMPTOMS AND DEPRESSED AFFECT AMONG NURSING PERSONNEL

In recent years the concept of stress and perception of stressful life events has provided a number of avenues for research. Several studies have attempted to relate stressful life events to a variety of physical and psychological symptoms (Bell, 1977¹, Dohrenwend and Dohrenwend, 1969, 1974,^{2,3,4} Holmes and Rahe, 1967,⁵ and Rahe, 1968).⁶ Other authors suggest stress is related to physiological dysfunctions, disease, mental disorders and socially pathological behavior (Scotch and Levine, 1970).⁷

Weiman (1977)⁸ notes that although occupational stress, defined as the sum total of factors experienced in relation to work which affects the psychosocial and physiological homeostasis of the worker, has never been classified as a public health problem, it may be one of America's most significant causes of illness. He observes individual factors related to an occupation may be termed stressors and that stress is the individual worker's reaction to those stressors. In conjunction with this notion, Gross (1970)⁷ agrees that stress is a social psychological phenomena - a matter of the relation between an individual and the structure within which he finds himself.

Intensive care units may be considered highly stressful places to work. Stressors and stress may be particularly great for nurses in these units. Most of the studies in intensive care settings have focused on the stress levels of the patient, and only recently have studies begun to focus on stressors and stress experienced by the staff members of these units

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1. Bell JM: Stressful life events and coping methods in mental illness and wellness behaviors. *Nursing Research*, Vol 26, No 4:118-126, 1977.
 2. Dohrenwend BP and Dohrenwend B: Stressful life events: Their nature and effects. New York. John Wiley, 1974.
 3. Dohrenwend, BS: Social status and stressful life events. *J of Personality and Social Psychology*, 28:225-235, 1973.
 4. Dohrenwend, BP: The social psychological nature of stress. *J of Abnormal and Social Psychology*. 62:294-302, 1961.
 5. Holmes TH and Rahe RH: The social readjustment rating scale. *J of Psychosomatic Research* 11:213-218, 1967.
 6. Holmes T and Masuda M: Life change and illness susceptibility in stressful life events: Their nature and effects (Ed) Dohrenwend B and Dohrenwend B. New York, John Wiley, 1974.
 7. Gross E: Work, organization and stress. Social stress (Ed) Levine and Scotch, Chicago, Aldine Publishers, 1970.
 8. Weiman C: A study of occupational stressors and incidence of disease/risk. *J of Occupational Medicine* 13:119-124, Feb 1977.

(Cassem and Hackett, 1975,⁹ Vreeland, 1969)¹⁰. Some of the stressors associated with intensive care units are: overload of patients, constant sensory stimuli, pain, death, tension, and anxiety (Schnaper and Cowley, 1976).¹¹ While most of the studies have focused on coronary care and general medical-surgical units, very little literature exists concerning burn care treatment units. Burn injuries and burn care problems are frequent, multiple and complex (Feller and Archambeault, 1973).¹² Many burn victims spend their initial treatment period in some form of intensive care facility. There are also a great number of infections, deaths and painful procedures associated with burn injuries. These procedures and events may be as stressful for staff members of the unit as they are for the patients.

What then, is the nature of occupational stress associated with burn-care nursing? How might this stress affect the psychosocial and physiological homeostasis of the nursing personnel? Quinby and Bernstein (1971)¹³ note that caring for burned children poses problems in terms of personal¹⁴ and professional identity for nurses. In addition, Bernstein (1976)¹⁴ has documented feelings of confusion, ambivalence, frustration, guilt, anger and hostility in nursing personnel who work with burned children. Coupled with these feelings, Bernstein¹⁴ (1976) notes that some of the staff suffer from nightmares and dreams of destruction. One could hypothesize from this that occupational stress associated with burn wound nursing has many psychosocial and physiological ramifications. Accordingly, this study proposes to analyze the relationship of perceived stressful life events, psychophysiological symptoms and depressed moods of nursing persons who work in an intensive care burn unit.

The null hypothesis of this study which will be tested at an alpha level of .05 is that there is no significant correlation between symptoms, depression and life change units in this sample of burn unit nursing personnel.

9. Cassem N and Hackett T: Stress on the nurse and therapist in the intensive-care unit and the coronary-care unit. *Heart and Lung* 4:252-259, March-April, 1975.

10. Vreeland R and Ellis G: Stresses on the nurse in an intensive care unit. *JAMA* 208: No 2, April 14, 1969.

11. Schnaper N and Cowley R: Overview: Psychiatric sequelae to multiple trauma. *Amer J of Psychiatry* 133:883-890, August 1975.

12. Feller I and Archambeault C: Nursing the burned patient. Ann Arbor, Michigan Institute of Burn Medicine, 1973.

13. Quinby I and Bernstein N: Identity problems and adaptation of nurses to severely burned children. *Amer J of Psychiatry* 128:90-95, Jul 1971.

14. Bernstein N: Emotional care of the facially burned and disfigured. Boston, Little Brown and Company, 1976.

METHOD

SUBJECTS:

A sample of convenience consisted of 47 members of the nursing staff of the U. S. Army Institute of Surgical Research at Brooke Army Medical Center. The duty roster indicates there are 68 nursing persons assigned to the unit. Fifty-three questionnaires were returned to the investigator. It should be noted some members of the staff were on leave and others were on temporary duty assignments elsewhere. Of the 53 questionnaires returned, 6 were discarded due to incomplete responses.

Data gathered from 47 of the nursing personnel were analyzed. Twenty-nine males and 18 females participated in this study. Thirty-nine of the respondents were military personnel, 8 were civil service employees. Among the group, 14 were Registered Nurses and 33 were Licensed Practical Nurses. The average age of the subjects was 29.9 years. The average education level was 14.21 years. The mean number of months individuals had been associated with the unit was 28.75 months. Characteristics, means and standard deviations of the nursing personnel are summarized in Table 1.

INSTRUMENTS AND DESIGN

A 91-item paper and pencil questionnaire was utilized on this study. The questionnaire was composed of 3 instruments. The first instrument was a 22-item screening inventory of psychophysiological symptoms indicating impairment, developed by Langer (1962).¹⁵ The inventory was utilized originally to provide a rough indication of where people lie on a continuum of impairment in life functioning due to very common types of psychiatric symptoms. The 22-item screening score was developed during the course of the Midtown Study of mental disorders in Manhattan, New York City. Based on the Midtown Study of mental disorders, the instrument was validated on a "known well" group and "known ill" group. The 22 symptoms clearly distinguished between the patient group and the "known well" group. The 22 items all discriminated between the ill and well groups at the .01 confidence level or better (Langer, 1962).¹⁵

The Social Readjustment Rating Scale (SRRS) developed by Holmes and Rahe (1967),⁵ the second instrument used, has been used in a number of other studies. The scale lists 43 life events which are either indicative of or require some change in the life of an individual. Each event has been assigned a life change unit value depending on how much social readjustment was judged necessary to adapt to each event. Holmes and Rahe (1967)⁵ found that scores of 150-199 life change units (LCU) indicate mild stress, 200-299 LCU indicate moderate stress and scores of 300 and above indicate high life stress. The tool has been used as a predictor of illness (Rahe 1968). Several studies have reported high

15. Langer TS: A twenty-two item screening score of psychiatric symptoms indicating impairment. J of Health and Human Behavior 3:269-276, 1962.

correlations confirming the validity of the instrument (Holmes & Masuda, 1974).⁶

The third component of the questionnaire was a 20 item scale designed by the Center for Epidemiological Studies (CES) and utilized in a Community Mental Health Assessment project (Markush & Fevero, 1974).¹⁶ The Community Mental Health Assessment project was a division of the Extra-mural Research Programs of the National Institute of Mental Health. The Center for Epidemiological Studies Depression (CES-D) scale was developed from five previously developed scales which included a depression inventory by Beck (1967)¹⁷ and items from the depression scale of the M.M.P.I. (Minnesota Multiphasic Inventory) and was pretested on clinical populations in Philadelphia.

Questionnaires were distributed to nursing personnel at the U.S. Army Institute of Surgical Research Burn Unit by the investigator. Subjects were asked to voluntarily participate in a research project investigating "stress on nurses."

Respondents were to return the questionnaires to the investigator at their earliest convenience. Although respondents remained anonymous the questionnaire required the following demographic data: sex, age, professional status (R.N./L.P.N.), military or civil service affiliation, educational level, and number of months on the burn unit.

After responses were tabulated each subject received 3 scores. The score for the 22-item screening inventory was based on a simple count of positive responses as described by Langer (1962).¹⁵ The life change unit scores were derived from data provided by Holmes & Rahe (1967),⁵ and the depression score was the sum of weighted responses as described by Markush & Favero (1974).¹⁶ A computer program of stepwise multiple linear regression analysis was used to analyze the data.

RESULTS

Pearson Product Moment Correlations generated by the computer program resulted in significant positive correlations ($p < .05$ with 45 Degrees of Freedom) between the scores at the three instruments. As shown in Table 2, the correlation between the Symptoms and Depression measures was .656. The correlation between the Symptoms and Life Change measures was .334. The correlation between the Depression and Life Change measures was .309. Accordingly the null hypothesis was rejected.

16. Markush R and Favero R: Epidemiological assessment of stressful life events, depressed mood and psychophysiological symptoms in stressful life events their nature and effects. Dohrenwend and Dohrenwend (Ed.) New York. John Wiley, 1974.

17. Beck AT: Depression: Clinical, experimental and theoretical aspects. New York. Harper and Row, 1967.

The mean scores for Psychophysiological Symptoms, Life Change Units and Depression measures are shown in Table 3.

The mean Symptom score was 3.44 with a standard deviation of 3.06. The mean number of Life Change Units for this sample was 141.12 (S.D.=118.33) and the mean Depression score was 17.66 (S.D.=6.92).

Table 4 illustrates the percentages of Psychophysiological Symptoms, Life Change Units, and Depressed mood in this sample of nursing personnel. Forty-six and eight tenths percent (22) of the respondents were assessed as moderately depressed (scores of 6-15 on the CES-D scale), 53.19 percent (25) fell into the category of high depressed mood (scores of 17-60 on the CES-D scale).

In terms of psychophysiological symptoms 10.6 percent (5) received low scores (0), 48.9 percent (23) received middle scores (1-3), and 40.42 percent (19) received high scores (4-6) as defined by Langer (1962).¹⁵

Life Change Unit scores were less than 150 on 29 or 61.72% of the personnel. The remaining eighteen individuals were evenly distributed in the categories of mild, moderate and high life crises with 6 or 12.76% assigned to each.

DISCUSSION

The results of this preliminary analysis suggest there is significant correlation between psychophysiological symptoms and depression in this sample of burn unit nursing personnel. Furthermore there is a significant correlation among psychophysiological symptoms, life change units and depression. Further analysis may tease out other variables and relationships which will yield other significant correlations. This study corroborates some of the conclusions of other investigations (Dohrenwend, 1971, Holmes & Markush, 1974, Markush & Favero, 1974),¹⁶ in that significant associations between depressed mood and symptom scores are noted.

While depression is identified as an affective state among this sample of burn unit nursing personnel, more specific methods must be developed in order to assess its etiology. Schnaper and Cowley (1976)¹¹ suggest frustration is manifested by fatigue, forgetfulness, depression and guilt in nurses who deal with multiple trauma. Is frustration the basic cause of burn nurses' depression? Kline (1974)¹⁸ has discussed the chemical and physiological changes that occur in patients with depression. Weiman (1977)⁸ suggests occupational adversity can result in physiological responses which may become irreversible if the noxious stimulus never changes or if adaptation does not occur. Further study is needed to determine the physiological manifestations of depression

18. Kline N: From Sad to Glad. New York: Putnam and Sons, 1974.

in burn unit nurses and which physiologic correlates are related to the specific stressors of burn nurses?

This study indicated 46.8 percent (22) of the sample were moderately depressed, 53.19 percent (25) fell into the high range of depression. It will require other studies to identify means to alleviate this syndrome and assess the effectiveness of such means and the duration of relief. Cassems & Hackettt (1975)⁹ suggest that group crisis meetings for personnel help alleviate much of the stress associated with intensive care units. In these meetings the physiological manifestation of stress and the feeling generated by it must be identified and acknowledged, shared, examined, and integrated. Such meetings would perhaps dissipate some of the depression and offer nursing persons an avenue for ventilation. Cassem & Hackett further suggest that those who work in intensive care settings risk their own lives, feelings, esteem and self-respect. Risk can produce growth; however, this risk can also produce a dehumanization of the nurse as well as the patient and risk limits must be defined.

Further exploration of occupational stress of burn nurses is needed. It is advantageous for nursing administrative personnel and medical personnel to be aware of the psychosocial and physiological homeostasis of their workers. Systematic study may reveal that homeostasis may not be the predominate state in which people function, and identification of stressors may lead to control and treatment of psychosocial and physiological disturbances. Interpretation of these data must be tempered by the realization that this study does not differentiate between cause and effect. The alternative hypothesis that depressed people or people predisposed to depression choose to work in intensive care settings must also be rigorously examined.

PRESENTATIONS AND PUBLICATIONS

Pesut DJ, Carlson LL, Relationship between psychological symptoms, stressful life events, and depressed mood in burn nursing personnel. Presented at the Tenth Annual Meeting of the American Burn Association, Birmingham, Alabama, 30 March 1978.

TABLE 1

CHARACTERISTICS OF THE BURN UNIT NURSING PERSONNEL
(N = 47)

Sex:			29 Males 18 Females
Age:	Mean	=	29.9 years
	Standard Deviation	=	8.12
Educational Level:	Mean	=	14.21 years
	Standard Deviation	=	2.06
Military:			39
Civil Service:			8
Registered Nurses:			14
Licensed Practical Nurses:			33
Months on unit:	Mean	=	28.75 months
	Standard Deviation	=	

TABLE 2

PEARSON CORRELATION OF PSYCHOPHYSIOLOGICAL SYMPTOMS,
LIFE CHANGE UNITS, AND DEPRESSION SCORES

Measure	Correlation
Symptoms and Depression	.656*
Symptoms and Life Change	.334*
Depression and Life Change	.309*

*Significant at $p < .05$ 45 Degrees of Freedom

TABLE 3

MEAN SCORES AND STANDARD DEVIATIONS OF PSYCHOPHYSIOLOGICAL, LIFE
CHANGE, AND DEPRESSION MEASURES OF BURN UNIT NURSING PERSONNEL
(N = 47)

	<u>Mean</u>	<u>Standard Deviation</u>
Symptoms	3.44	3.06
Life Change	141.12	118.33
Depression	17.66	6.92

TABLE 4

PROPORTIONS OF PSYCHOPHYSIOLOGICAL SYMPTOMS, LIFE CHANGE
UNITS AND DEPRESSION IN BURN UNIT NURSING PERSONNEL

	Low		Middle		High		Total	
	N	%	N	%	N	%	N	%
A. Psychophysiological Symptoms	5	10.6	23	48.9	19	40.42	47	100.
B. Life Change Units	6	12.76	6	12.76	6	12.76	18	38.28*
C. Depressed Mood	0		22	46.8	25	53.19	47	99.99

*61.72 percent (29) had LCU < 150

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a		2. DATE OF SUMMARY ^a		3. REPORT CONTROL SYMBOL (DD FORM 1498, 1 NOV 68)	
4. DATE PREV SUMMARY ^a		5. KIND OF SUMMARY		6. SUMMARY SCTY ^a		7. WORK SECURITY ^a		8. REGRADING ^a	
77 10 01		D. CHANGE		U		U		NA	
9. NO./CODES ^a		10. PROGRAM ELEMENT		11. PROJECT NUMBER		12. TASK AREA NUMBER		13. WORK UNIT NUMBER	
A. PRIMARY		61102A		3S161102BS05		00		090	
B. CONTRIBUTING									
C. CONTRIBUTING									
14. TITLE (Provide with Security Classification Code) ^a									
(U) Alteration of Host Resistance in Burned Soldiers (44)									
15. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a									
003500 Clinical Medicine									
16. START DATE			17. ESTIMATED COMPLETION DATE			18. FUNDING AGENCY		19. PERFORMANCE METHOD	
76 10			Cont			DA		C. In-House	
20. CONTRACT GRANT									
Not Applicable									
21. A. DATES/EFFECTIVE:				22. B. EXPIRATION:				23. C. FISCAL YEAR	
A. NUMBER ^a				B. TYPE				C. FUNDING ESTIMATE	
C. TYPE				D. AMOUNT:				E. PROFESSIONAL MAN YRS	
F. KIND OF AWARD:				G. F.CUM. AMT.				H. FUNDS (in thousands)	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION					
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research					
ADDRESS: Ft Sam Houston, Texas 78234				ADDRESS: Ft Sam Houston, Texas 78234					
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)					
NAME: Basil A. Pruitt, Jr., COL, MC				NAME: Albert T. McManus, Jr, CPT, MSC					
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-3411					
21. GENERAL USE				22. ASSOCIATE INVESTIGATORS					
FOREIGN INTELLIGENCE NOT CONSIDERED				NAME:				DA	
23. KEY WORDS (Provide SSAN with Security Classification Code)									
(U) Opportunistic pathogens; (U) Microbial virulence; (U) Tissue spreading factors; (U) Rat model; (U) Infection; (U) Neutrophil function; (U) Metabolism; (U) Immunostimulants									
24. TECHNICAL OBJECTIVE (25. APPROACH, 26. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code))									
25. (U) To define the basis of infection susceptibility in burned soldiers. To identify specific white blood cell dysfunction and establish the metabolic basis for such. To determine the effect of antibiotics on neutrophil function. Establish a burned rat model of granulocyte dysfunction. Examine the effect of immunostimulant drugs and metabolism altering drugs on rat granulocyte function. Examine tissue spreading factors of opportunistic pathogens that may accentuate defects in host phagocyte mobilization.									
26. (U) The high susceptibility of burned rats to Pseudomonas infection will be investigated. Rat granulocyte function will be examined. The <u>in vitro</u> effects of antibiotics and stimulants will be examined in cells from burned humans.									
27. (U) 7710 - 7809 An <u>in vivo</u> defect in chemotaxis in burned rats has been established and conditions of purification and isolation of peripheral rat granulocytes have been established. Pseudomonas motility has been established as an important virulence factor in rat burn wound sepsis. The 60% burned rat has been found to be susceptible to infection with a fungal (Mucor) strain isolated from a fatal human burn wound sepsis.									

^a Available to contractors upon or (author's approval).

DD FORM 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DO FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigator:

Albert T. McManus, Jr., Captain, MSC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

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Reports Control Symbol MEDDH-288(R1)

A burned rat model of altered granulocyte function has been previously reported (1). The 350 gram Charles River rat with a 60% full thickness scald injury has hematological changes that closely mimic burned man (2). Significant evaluation in circulating absolute neutrophilic counts were observed on day one and again on day four through 21 days post injury. Despite elevated circulating neutrophil count, burned animals were unable to muster a normal inflammatory response to the intraperitoneal injection of sterile casein. It appeared that these animals were in a depressed inflammatory state rather than a neutropenic state. The mechanisms of depressed neutrophil activity is being investigated under a separate project (3).

The inability of the burned host to mobilize phagocytes into contaminated wounds would seem an obvious advantage to opportunistic pathogens (4,5). If an opportunistic pathogen has active but conditionally invasive mechanisms, depression of host phagocyte mobilization could allow selective infections with parasites sensitive to a normal inflammatory response.

1. McManus AT: Alteration of host resistance in burned soldiers. USAISR Annual Report, p. 49, FY 1977.

2. Sevitt S: Eosinophil and other leukocyte changes in burned patients with special reference to adrenocortical activity. Brit Med J 1:976, 1951.

3. McManus AT, Mason AD Jr: Laboratory investigation of the mechanisms of acquired leukocyte dysfunction following thermal injury of burned soldiers. USAISR Annual Report, FY 1978.

4. McCabe WP, Rebeck JW, Kelly AP Jr, Ditmars DM: Leukocytic response as a monitor of immunodepression in burned patients. Arch Surg 106:155, 1973

5. Warden GD, Mason AD Jr, Pruitt BA Jr: Evaluation of leukocyte chemotaxis in vitro in the thermally injury patient. J Clin Invest 54:1001, 1974.

The hypothesis that bacterial mobility was a component in experimental *Pseudomonas* burn wound sepsis has been tested. The results are described in the attached report.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS
-- BACTERIAL MOTILITY: A COMPONENT IN EXPERIMENTAL
PSEUDOMONAS AERUGINOSA BURN WOUND SEPSIS

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ABSTRACT

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Mutants which do not spread in soft agar were derived from a rat burn wound virulent strain of Pseudomonas aeruginosa. When inoculated onto rat burn wounds, these motility altered strains had significantly reduced virulence. Control agar spreading isolates that had undergone the same manipulations were found to have maintained virulence. Other than motility, no other parental characteristic was found to be altered. The nonspreading isolates were virulent when inoculated below the burn wound. It is concluded that motility is an important factor in experimental burn wound sepsis.

Rat model
Endotoxemia
Invasion

ALTERATION OF HOST RESISTANCE IN BURNED
SOLDIERS -- BACTERIAL MOTILITY: A COMPONENT
IN EXPERIMENTAL PSEUDOMONAS AERUGINOSA
BURN WOUND SEPSIS

The characteristic factors which allow Pseudomonas aeruginosa to be a common and frequently successful opportunistic pathogen are not well defined. Investigations of possible pathogenic mechanisms have emphasized the reproduction of the clinical toxicity which frequently accompanies severe pseudomonas infection (1,7). Several varieties of toxic extracellular products including toxins, hemolysins and proteolytic enzymes have been isolated (5,8,9). The bioassay of the toxicity of these materials has generally been accomplished in mice. The roles of specific factors in the process of infection, per se, have only recently been addressed. Injection of pseudomonas strains with known in vitro capacity to produce specific toxins has implicated their importance in vivo (11,15). Such injections of organisms, however, would seem to avoid the requirement that a virulent strain colonize and invade the host.

We have examined bacterial motility as a requirement for virulence in a rat model of progressive infection following burn surface inoculation. The approach was to test for loss of virulence in nonmotile isolates derived from a virulent motile parent strain.

-
1. Alexander JW: Control of infection following burn injury. Arch Surg 103: 435-441, 1971.
 7. Moncrief JA & Teplitz C: Changing concepts in burn wound sepsis. J Trauma 4: 233-245, 1964.
 5. Liu PV: Extracellular toxins of Pseudomonas aeruginosa. J Infect Dis 130: Suppl S-94, 1974.
 8. Muszynski Z: Enzymatic and toxinogenic activity of culture filtrates of high and low virulence strains of Pseudomonas aeruginosa on mice. Pathol Microbiol 39: 135-147, 1973.
 9. Pavlovskis OR & Shackelford AH: Pseudomonas aeruginosa exotoxin in mice: Localization and effect on protein synthesis. Infect Immun 9: 540, 1974.
 11. Stieritz DD & Holder IA: Experimental studies of the pathogenesis of Pseudomonas aeruginosa infection: Evidence for the in-vivo production of a lethal toxin. J Med Microbiol 11: 101-109, 1978.
 15. Wretling B & Kronevi T: Experimental infections with protease-deficient mutants of Pseudomonas aeruginosa in mice. J Med Microbiol 11: 145-154, 1978.

MATERIALS AND METHODS

Rat Burn Model - The Walker-Mason rat scald technique (14) was used. A 30% full thickness burn injury was inflicted on 350 g rats anesthetized with sodium pentobarbital (25 mg/kg). Thermal exposure was a 10 second exposure of the shaved dorsum to boiling water. No fluid resuscitation was administered.

Parental Organism - Strain 59-12-4-4, a human blood isolate, was used. The rat virulence of this strain has been previously reported (13,6).

Mutagenesis and Nonmotile Clone Isolations - The parental strain was exposed for one hour to nitrosoguanidine at a dose of 0.1 mg/ml in 0.1 M citrate buffer, pH 5.0, at 37°C. Cells were then washed in Trypticase soy broth (TSB) and incubated for 12 hours at 35°C in a shaking water bath. The culture was then washed in TSB and serial 10-fold dilutions were prepared in TSB. The first 4 dilutions were plated (0.1 ml/plate) into 10 ml of one-third strength TSB containing 1% NaNO₃ and 0.5 agar. Fifty plates were prepared from each dilution and incubated at 35°C for 24 hours. Following incubation, plates were examined for non-"haloed" colonies. Sixteen non-spreading colonies were isolated. As mutagenesis procedural controls, 5 spreading clones from the same plates were isolated. The isolated non-spreading clones were examined by hanging drop and incubated in TSB overnight. Following incubation, the isolates were examined for motility by hanging drop observation and by stabbing semisolid medium (0.5% agar).

Virulence Testing - Parental, nonmotile and control isolates were grown overnight in shaking TSB cultures. Inocula were diluted in TSB to 10⁸ cfu/ml and 1 ml was spread over the scald wounds.

Nonmotile isolates were also examined for virulence when inoculated below the burn wound. The inocula were 10⁸ cfu (1 ml) given as 4 injections of 0.25 ml.

Growth Comparisons - Strains were compared for relative growth rates in shaking TSB cultures. Growth was measured by plate counting as a function of culture age.

Protease Production - Total protease activity was measured in culture filtrates of 24 hour cultures grown in dialyzed TSB. The growth medium was

14. Walker HL & Mason AD, Jr: A standard animal burn. J Trauma 8: 1049-1051, 1968.

13. Walker HL, Mason AD, Jr & Raulston GL: Surface infection with *Pseudomonas aeruginosa*. Ann Surg 160: 297-305, 1964.

6. McEuen DD, Blair P, Delbene VC & Eurenus K: Correlation between pseudomonas burn wound infection and granulocyte antibacterial activity. Infect Immun 13: 1360-1362, 1976.

prepared by dialyzing 3X concentrated broth against 2 volumes of deionized water. Filtrates were examined for total protease activity using commercially obtained (Sigma) hide powder azure as a substrate (10).

Electron Microscopy - Suspensions of agar grown colonies were prepared in water and collected on Formvar grids. Grids were air dried and shadowed with platinum.

Examination for Metabolic Alterations - Nitrosoguanidine mutagenesis has been reported to induce high frequency of auxotrophic and cell membrane mutations (4). Motility variants were examined for auxotrophy by measuring growth on minimal agar (3). Alterations in cell somatic antigens were examined serologically (Difco typing set). Strain metabolic activities were examined using the API (R) taxonomic system. Changes in strain antibiograms were examined by the Kirby-Bauer technique (2).

RESULTS

Strain Isolations - Following TSB broth subculture, only 5 non-"haloed" isolates remained nonspreading in 0.5% agar. The strains were designated M-5, M-6, M-9, M-10, and M-13. Spreading control isolates were designated W-1, W-2, W-3, W-4, and W-5. All strains were then stored at -80°C in sterile milk. Further attempts to revert the nonspreading strains by static subculture were unsuccessful. Strain M-13 was found to be nonspreading in agar concentrations as low as 0.3%, yet was motile by hanging drop in liquid culture. Organisms removed from nonspreading subsurface colonies were motile when mounted in water. Strain M-6 was found to have a low (approximately 1/1000 cells) frequency of liquid-motile cells, but no agar-spreading clones could be demonstrated. Figure 1 shows that upon electron microscopic observation, strains M-5, M-9, and M-10 were non-flagellated. Strains M-6, M-13, and the parent were monotrichous.

Rat Burn Wound Virulence - Mortality data are presented in Table I. Animals inoculated with nonspreading isolates had significantly increased survival when compared with those receiving control and parental strains

10. Rinderknecht H, Geokas MC, Silverman P & Haverback BJ: A new ultrasensitive method for the determination of proteolytic activity. Clin Chim Acta 21: 197-203, 1968.

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3. Clowes RC & Hayes W: Experiments in Microbial Genetics. John Wiley & Sons, Inc. (New York), 1968, p. 185.

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Fig. 1. Strain designations are from left to right: parent, M-5, M-6, M-9, M-10, and M-13. The bar represents 1 micron.

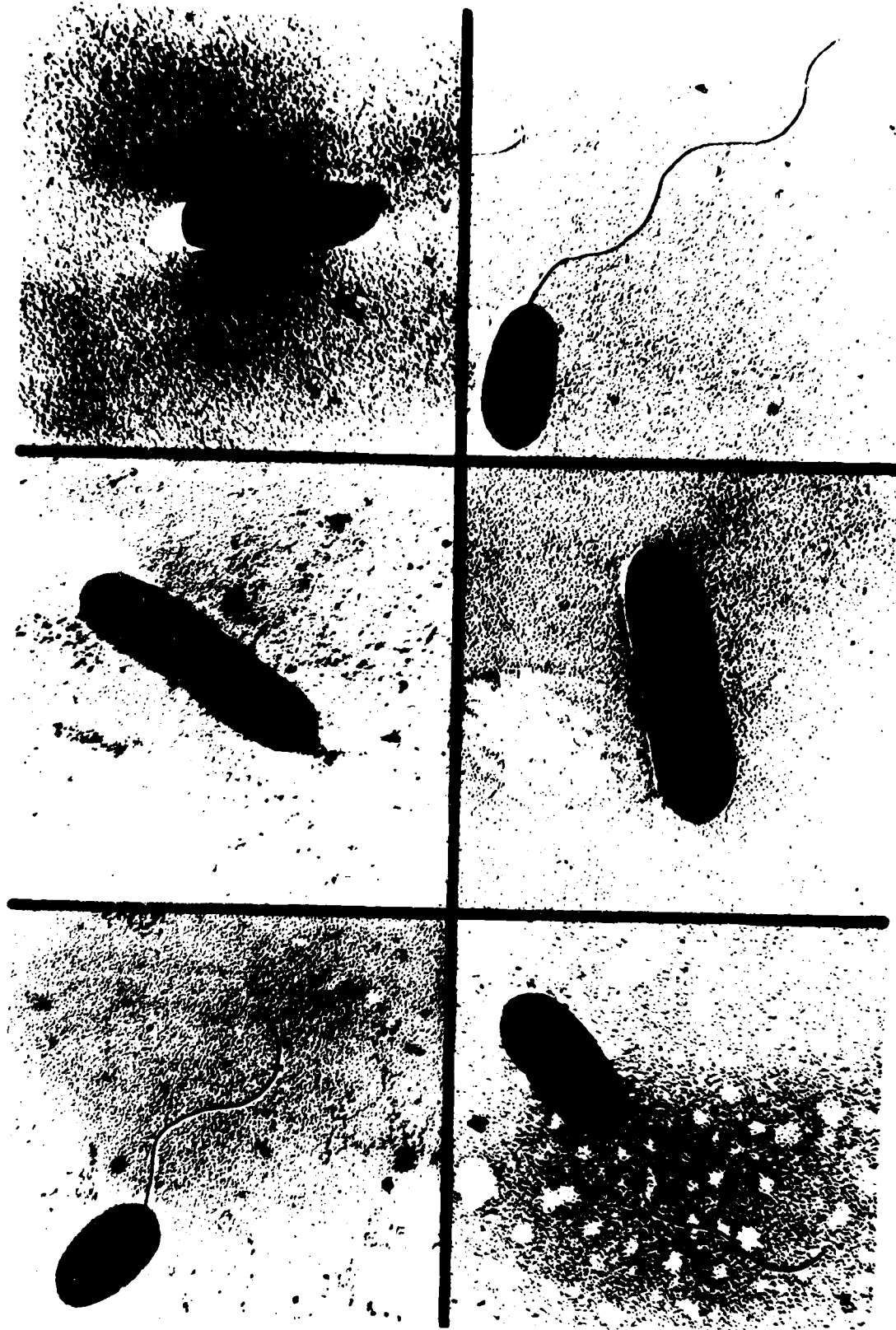


Table I. Rat Burn Wound Virulence^a

	Days Postburn Inoculation																				Dead
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21/28
M-5									1		2						1	1			5/10
M-6												3		1	1	1	1				7/10
M-9																		1			1/10
M-10													1	1	1						3/10
M-13													1				1	1			3/10
Total																					19/50
W-1				1	1		1	1	1	1		3									9/10
W-2				1	1					1	1		1			2	2	1			10/10
W-3			2				1	1	1				1		1	1	1	1			10/10
W-4							1		1			3	3			1		1			10/10
W-5					1	1										2	3				9/10
Total																					48/50
Parent	1						4	2	3	1	2	5	1		1						20/20

^a Mortality results following 10^8 cfu inoculation of burn wound surface of 30% scalded 350 g rats.

($p < 0.01$). Chi square analysis was used. Additionally, nonsurvivors in the nonspreading groups had an extended time to death when compared with control groups ($p < 0.01$).

Injection of the nonspreading isolates into the burn resulted in increased mortality (33/50) when compared with the mortality observed with surface inoculation (19/50) ($p < 0.01$). As a group, injected nonspreading isolates did not produce mortality equal to that observed with surface inoculation of control organisms ($p < 0.01$).

Protease Production - Data are presented in Table II. Analysis of variance showed no difference between the motile and nonmotile groups, but within the nonmotile group, protease production by strain M-9 was significantly lower than the mean of the remaining strains ($p < 0.01$).

Table II. Total Protease Activity^a

<u>Nonmotile Clones</u>		<u>Motile Clones</u>	
M-5	13.49 \pm 0.47	W-1	13.85 \pm 0.49
M-6	16.69 \pm 1.48	W-2	12.94 \pm 1.34
M-9	7.59 \pm 1.94	W-3	14.09 \pm 1.29
M-10	15.33 \pm 2.45	W-4	13.58 \pm 2.5
M-13	13.14 \pm 1.22	W-5	17.02 \pm 4.37
Parent Strain			
12.96 \pm 0.31			

^a Total protease activity in culture filtrates following 24 hour growth. Data presented as means of duplicate samples run on triplicate cultures. Data are in trypsin activity equivalents (ug/ml) at pH 7.8 using hide powder azure substrate.

Growth, Metabolic Activity, Serotype, and Antibiotic Sensitivity Comparisons - Neither nonspreading isolates nor control cultures had auxotrophic requirements; all strains grew on minimal agar. API (R) codes were identical except for motility between parent and nonspreading strains. Regression analysis of the exponential growth curves showed no significant difference in growth rates, nor was any significant difference found between maximum stationary growth levels. Serotypes and antibiograms were unaltered.

DISCUSSION

Teplitz has described the histopathologic similarity between experimental pseudomonas burn wound sepsis and the human disease (12). In his

12. Teplitz C, Davis D, Mason AD, Jr & Moncrief JA: Pseudomonas burn wound sepsis. I. Pathogenesis of experimental pseudomonas burn wound sepsis. J. Surg Res 5: 200-216, 1964.

elegant study, Teplitz followed the bacteriologic course from surface inoculation to death. The infection clearly progressed from colonization of the superficial eschar through massive accumulation of bacteria in nonviable tissue to invasion of viable hypodermal and adjacent tissue with subsequent hemogenous spread and death. The progressively invasive nature of the infection prompted us to ask what role motility might play in this disease.

In this report, selection for loss of colony spreading in soft agar resulted in diminished virulence. The differences in flagellar morphology and protease production indicate that these isolates are not strains of an expanding single mutant clone. No other known parental markers were changed. Loss of virulence was directly related to loss of agar spreading; unless one assumes that agar spreading is linked with other unknown virulence characteristics of the parent, or that mutagenesis resulted in loss of other unrelated virulence linked markers which were randomly picked in the nonspreading strains and not in the motile controls. We feel that these alternatives are unreasonable. Bacterial motility is an important element in the pathogenesis of Ps. aeruginosa experimental burn wound sepsis. Additionally, these findings suggest bacterial motility as an important consideration in the development of antimicrobial and chemotherapeutic agents.

SUMMARY

Mutants which do not spread in soft agar were derived from a rat burn wound virulent strain of Ps. aeruginosa. When inoculated onto rat burn wounds, *these motility altered strains had significantly reduced virulence*. Control agar spreading isolates that had undergone the same manipulations were found to have maintained virulence. Other than motility, no other parental characteristic was found to be altered. The nonspreading isolates were virulent when inoculated below the burn wound. It is concluded that motility is an important factor in experimental burn wound sepsis.

PRESENTATIONS:

McManus AT: Decreased virulence in experimental burn wound sepsis associated with motility mutants of Ps. aeruginosa. Amer.Soc. Microbiol. Las Vegas, Nevada, 13-19 May 1978.

PUBLICATIONS:

McManus AT, Moody EEM, Mason AD, Jr: Bacterial motility: A component in experimental Pseudomonas aeruginosa burn wound sepsis. Submitted for publication.

PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, Military Burn Research

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS --
IMPORTANCE OF THE IMMUNE SYSTEM IN PSEUDOMONAS INFECTION.
A STUDY OF INFECTION RATES IN IMMUNE-DEFICIENT AND
IMMUNE-COMPETENT AKR-J MICE

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

Thomas W. Panke, M.D., Major, MC
Charles G. McLeod, Jr, Major, VC
Paulette C. Langlinais, M.S.
Arthur D. Mason, Jr, M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05-00, Military Burn Research

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Reports Control Symbol MEDDH-288(R1)

Since AKR-J mice develop a selective impairment in the immune system, an attempt was made to develop a model with a controlled hematogenous infection by Pseudomonas aeruginosa. In the initial test, quantified inocula of Ps. aeruginosa were injected intraperitoneally. An early high mortality rate was ascribed to endotoxemia since the mice died rapidly after inoculation and no evidence of hematogenous lesions were seen in the viscera.

Subsequently, skin flaps were raised on the back of these animals to produce nonviable tissue into which Ps. aeruginosa was inoculated. This technique resulted in hematogenous infection by Ps. aeruginosa with visceral dissemination and necrotizing lesions, but was not useful as an experimental model in our hands because of the unpredictable mortality rates when known challenge doses of Ps. aeruginosa were given to different groups of mice.

ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS -- IMPORTANCE OF THE IMMUNE SYSTEM IN PSEUDOMONAS INFECTION. A STUDY OF INFECTION RATES IN IMMUNE-DEFICIENT AND IMMUNE-COMPETENT AKR-J MICE

Prior to topical antibiotic therapy, invasive burn wound infection by Ps. aeruginosa was a very common pathway for the development of septicemia and subsequent demise of many burn patients. Silvadene and Sulfamylon burn creams have significantly decreased this incidence (1). However, the persistence of a high fatality rate due to invasive burn wound infection by Ps. aeruginosa indicates a vital need to study the mechanisms responsible for host defense.

There is abundant literature documenting the abnormal alterations in the immune system of the burn patient. Depressions of both the "B" and "T" lymphocyte systems have been reported (2, 3, 4, 5). Further complicating this is the demonstration of an ineffective and probably overwhelmed reticuloendothelial system, abnormal granulocyte responses to stimuli (6, 7, 8) and abnormal granulocyte metabolism (9, 10). The

1. Lindberg RB, Pruitt BA Jr, Mason AD Jr: Topical chemotherapy and prophylaxis in thermal injury. *Chemotherapy* 3:351-359, 1976.
2. Arturson G: Serum immunoglobulin levels in severe burns. *Research in Burns*, Eds Matter P, Barclay K, Konickova Z. Hans Huber Publ: Bern, Stuttgart, Vienna, 1971, pp 489-495.
3. Fumarola D, Cagnazzo G: Investigations on the response to PHA of lymphocytes from burned patients. *Research in Burns*, Eds, Matter P, Barclay TL, Konickova Z; Hans Huber Publ: Stuttgart, 1971, p 509.
4. Leguit P Jr: A study on the appearance of auto-immune phenomena in burn patients and its clinical relevance. *Basic Problems in Burns*, Eds, Vrabec R, Konickova Z, Moserova J, Springer Verlag, NY, Berlin 1975, pp 145-149.
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7. Warden GD, Mason AD Jr, Pruitt BA Jr: Evaluation of leukocyte chemotaxis in vitro in thermally injured patients. *J Clin Invest* 54:1001-1004, 1974.
8. Warden GD, Mason AD Jr, Pruitt BA Jr: Suppression of leukocyte chemotaxis in vitro by chemotherapeutic agents used in the management of thermal injuries. *Ann Surg* 181:363-369, 1975.
9. Gershwin RJ, Gershwin ME, Steinberg AD, Ahmed A, Ochiai T: Relationship between age and thymic function in the development of leukemia in AKR mice (39406). *Proc Soc Exp Biol Med* 152:403-407, 1976.
10. Heck E, Browne L, Curreri PW, Baxter CR: Evaluation of leukocyte function in burned individuals by in vitro oxygen consumption. *J Trauma* 15:486-489, 1975.

problem of determining the importance of each aspect of body defense mechanisms is all but insolvable.

The utilization of animal models with selected abnormalities is a possible approach to study sequentially the response to pseudomonas infections. Since the immune system undoubtedly plays a very important role in defense against the infections, the AKR-J strain of mouse was chosen as a test animal. AKR-J mice spontaneously develop a lymphoma of the thymus gland (11). During the evolution of this lymphoma, sequential and progressive deficiencies in the "T" and "B" (12, 13) lymphocyte systems occur. The AKR-J strain thus appeared to be a model in which to study the relative importance of the immune system in Pseudomonas infections.

METHODS

Syngeneic AKR-J mouse lymphoma was subcutaneously transplanted into AKR-J mice. These animals served as the experimental model.

RESULTS

1. Intraperitoneal injection of Ps. aeruginosa:

Initial experiments were directed at demonstrating an LD 50 of Ps. aeruginosa injected intraperitoneally. The inocula contained 10^3 to 10^8 microorganisms. All animals with 10^8 and most animals with 10^7 microorganisms died within 24 hours. The remainder of the animals survived. With the exception of petechiae, no lesions were seen; and specifically, infectious foci were not identified. These animals appeared to have died from an endotoxemia.

2. Inoculation of Ps. aeruginosa under skin flap:

Since the objective of this study was to produce an infectious disease and not an endotoxic death, it was elected to raise a dorsal skin flap on each animal, inoculate Ps. aeruginosa, and then close the skin flap over the inoculum.

11. McEndy DP, Boon MC, Furth J: On the role of thymus, spleen and gonads in development of leukemia in a high-leukemia stock of mice. Cancer Res 4:377-383, 1944.

12. Nagaya H: Thymus function in spontaneous lymphoid leukemia. 11 In vitro response of "preleukemic" and leukemic thymus cells to mitogens. J Immunol 111:1052-1060, 1973.

13. Vriend J (Anatomy Dept, Univ of TX at San Antonio): Unpublished data.

The results of this experiment are listed in Table I. This table summarizes the mortality rate for the various inocula of Ps. aeruginosa. For example, in Experiment #4, when 10^7 microorganisms were inoculated, six out of six animals died. When 10^6 microorganisms were inoculated, two of six animals died. With an inoculum of 10^5 , none of six animals died in three of the experiments.

Review of the table demonstrates that the LD 50 tended to occur between 10^5 - 10^6 microorganisms. However, the variation between experiments was so great (see Experiment #6) that no reliable point could be chosen for the LD 50.

TABLE I: INOCULATION OF PSEUDOMONAS AERUGINOSA UNDER SKIN FLAPS ON AKR-J MICE (ANIMALS DYING/TOTAL ANIMALS)

Experiment #	10^7	10^6	10^5	10^4	10^3
1		6/6	2/6		
2		6/6	4/6		
3			0/6	0/6	0/6
4	6/6	2/6	0/6		
5		3/6	0/6		
6		10/10	10/10	7/10	

DISCUSSION

Intraperitoneal inoculation of Ps. aeruginosa: Our attempt to induce Ps. aeruginosa septicemia by an intraperitoneal route was unsuccessful. Animals which expired did so within 1-3 days following challenge, but had no gross or microscopic hematogenous lesions. Death in these animals was attributed to endotoxemia.

Inoculation of Ps. aeruginosa below a skin flap: Because the goal of the project was to produce infectious hematogenous lesions in the viscera, the microorganisms were inoculated below a non-viable skin flap, surgically raised on the back of these mice. By elevating the skin flap, some tissue underwent necrosis and provided a more "natural" environment in which infection might develop. The results list mortality which basically appeared due to hematogenous Ps. aeruginosa with necrotizing lesions occurring in the lungs and occasionally in other tissues. However, quantification of the challenge dose of bacteria fatal for these mice demonstrated that the procedure was not useful as an experimental model in our hands. Precisely what causes the variation in mortality rate with given quantities of Ps. aeruginosa is unknown.

The enormous inequity of mortality rates in Experiment #6, compared to the above results, suggests an undetected illness in that group of mice.

PRESENTATIONS AND PUBLICATIONS

1. Panke TW, Langlinalis PC, Vriend J, McCue MJ: An animal model for childhood convoluted "T" cell lymphoma. Am J Pathol 92:595, 1978.
2. Panke T, Langlinalis P, Marmer D, Head D: An animal model for childhood convoluted T-cell lymphoma. Abstract: Anat Rec 190:502, 1978.
3. Poster Session: April 3-6, 1978, Vancouver, British Columbia, American Association of Anatomist Annual Meeting.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				DA OG 6975	78 10 01	DD FORM 1498, 1 NOV 68 10/11/1968	
1. DATE PREPARED 77 10 01	2. NAME OF SUMMARY D. CHANGE	3. SUMMARY TYPE U	4. WORK SECURITY U	5. PROGRAMING NA	6. INSTRUMENTATION NL	7. SPECIFIC DATA CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	8. LEVEL OF SUM A. WORK UNIT
10. NO. CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
A. PRIMARY	61102A	3S161102BS05	00	085			
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C. LOW PRIORITY							
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NAME* US Army Institute of Surgical Research				NAME* US Army Institute of Surgical Research			
ADDRESS* Ft Sam Houston, Texas 78234				ADDRESS* Ft Sam Houston, Texas 78234			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Provide with Security Classification Code)			
NAME Basil A. Pruitt, Jr., COL, MC				NAME* James F. Dorethy, MAJ, MC			
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11. CENTRAL USE				SOCIAL SECURITY ACCOUNT NUMBER			
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				NAME: Richard C. Treat, MAJ, MC			
				DA			
21. SUMMARY (Provide with Security Classification Code) (U) Humans; (U) Dogs; (U) Resuscitation fluids; (U) Echocardiography; (U) Burn injury; (U) Cardiac output; (U) Septic shock; (U) Cardiovascular hemodynamics							
22. TECHNICAL OBJECTIVE, 23. APPROACH, 24. PROGRESS (Provide individual paragraphs identified by number. Provide text of each with Security Classification Code.)							
23. (U) To evaluate systemic and pulmonary hemodynamic changes in burned soldiers and the influence of fluid resuscitation. To study noninvasively myocardial function in burned and burned-infected patients. To assess use of vasoactive agents in burned soldiers.							
24. (U) Hemodynamic flow and pressure changes are studied in burn patients during and after resuscitation. Myocardial function is studied by echocardiography. The effect of treatment on hemodynamic changes in burn patients with septic shock is assessed. Changes will also be studied in animal models.							
25. (U) 7710 - 7809 (I) Left ventricular (LV) performance in early burn shock was not abnormally affected by the type of fluid used for resuscitation. However, a colloid-containing solution more rapidly restored cardiac output and LV end-diastolic volume. Persistent burn shock was recognized in four patients resuscitated with a crystalloid solution. All improved hemodynamically with the addition of colloid. (II) LV performance during acute respiratory failure was studied in 30 patients. Sixteen were evaluated during therapy with continuous positive pressure ventilation. Echocardiography was able to discern which patients exhibited cardiovascular compromise. No evidence of myocardial depression was recognized during continuous positive pressure ventilation in patients with a decreased cardiac index. (III) LV function and cardiac output were measured in 10 thermally injured patients with septic shock. Three subclassifications were recognized: (1) hyperdynamic septic shock; (2) low output septic shock; and (3) myocardial abscess septic shock. The effects of dopamine on these subgroups were also evaluated.							

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PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORM 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: THE HEMODYNAMIC RESPONSE TO THERMAL INJURY IN BURNED
SOLDIERS -- I. SEQUENTIAL HEMODYNAMIC ALTERATIONS IN
SEVERE THERMAL INJURY IN THE MILITARY POPULATION --
COLLOID-CRYSTALLOID VERSUS CRYSTALLOID FLUID RESUS-
CITATION

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
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1 October 1977 - 30 September 1978

Investigators:

James F. Dorethy, M.D., Lieutenant Colonel, MC
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Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: THE HEMODYNAMIC RESPONSE TO THERMAL INJURY IN BURNED SOLDIERS -- I. SEQUENTIAL HEMODYNAMIC ALTERATIONS IN SEVERE THERMAL INJURY IN THE MILITARY POPULATION -- COLLOID-CRYSTALLOID VERSUS CRYSTALLOID FLUID RESUSCITATION

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

Investigators: James F. Dorethy, M.D., Lieutenant Colonel, MC
Richard C. Treat, M.D., Major, MC
Arthur D. Mason, Jr., M.D.,
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

This study evaluated 48 patients during postburn resuscitation. Forty-four of the patients formed the basis of an earlier report, while this communication reports the findings in four patients with persistent burn shock. The occasional postburn resuscitation failure has been attributed to a circulating myocardial depressant factor. However, direct evidence of decreased left ventricular (LV) performance has not been reported. All four patients had severe total body surface burns (values = mean \pm SEM, 80 ± 13 percent) and bronchoscopy evidence of inhalation injury, and received large volume crystalloid resuscitation (25.1 ± 3.9 L/24 hr Ringer's lactate). All required large amounts of sodium bicarbonate. Myocardial performance was measured by echocardiographic (ECHO) LV ejection fraction (EF) and mean velocity of diameter shortening (V_{cf} , circ/sec). The ECHO-EF was normal to increased, and the V_{cf} revealed hypercontractile myocardial performance. This occurred even with an inadequate cardiac index and severe metabolic acidosis.

Conclusions: Crystalloid resuscitation failure and persistent burn shock were manifested in this study by an inadequate cardiac index and a resistant metabolic acidosis. Myocardial performance was hypercontractile (increased ECHO V_{cf}), and no evidence of a serum myocardial depressant factor was found. These latter findings are consistent with those reported for the larger group of patients.

Postburn shock
Resuscitation fluids

Left ventricular function
Echocardiography

THE HEMODYNAMIC RESPONSE TO THERMAL INJURY IN BURNED SOLDIERS.
I. SEQUENTIAL HEMODYNAMIC ALTERATIONS IN SEVERE THERMAL INJURY
IN THE MILITARY POPULATION -- COLLOID-CRYSTALLOID VERSUS
CRYSTALLOID FLUID RESUSCITATION

Central hemodynamic changes immediately following severe thermal injury (greater than 40%) have been studied in the experimental animal (1-4) and man (5-7). Hypotension, severe metabolic acidosis, neurological impairment, renal failure, and cardiovascular collapse are manifestations of an immediate reduction of "effective" vascular volume. This circulatory state has been defined as "burn shock" (1,8). The exact pathophysiology is unclear (9), but is apparently due to the inability of central and peripheral cardiovascular compensatory mechanisms to maintain adequate cardiac output for oxygen delivery. Fortunately, this syndrome is rare, because of the increased utilization of aggressive early parenteral volume replacement. Rapid restoration of an "effective" cardiac output has almost eliminated renal failure and cardiovascular collapse as a cause of early post-burn death (10). However, 2% to 4% of severely burned individuals

1. Blalock A: Experimental shock. VII. Importance of local loss of fluid in production of low blood pressure after burns. Arch Surg 22:610-616, 1931.
2. Dobson EL, Warner GF: Factors concerned in the early stages of thermal shock. Circ Res 5:69-74, 1957.
3. Moncrief JA: Effect of various fluid regimens and pharmacologic agents on the circulatory hemodynamics of the immediate postburn period. Ann Surg 164:723-752, 1966.
4. Moylan JA, Mason AD Jr, Rogers PW, Walker HL: Postburn shock: A critical evaluation of resuscitation. J Trauma 13:354-358, 1973.
5. Unger A, Haynes BW Jr: Hemodynamic studies in severely burned patients. Surg Forum 10:356-361, 1960.
6. Pruitt BA Jr, Mason AD Jr, Moncrief JA: Hemodynamic changes in the early postburn patient: The influence of fluid administration and of a vasodilator (hydralazine). J Trauma 11:36-46, 1971.
7. Shoemaker WC, Vladeck BC, Bassin R, Printen K, Brown RS, Amato JJ, Reinhard JM, Kark AE: Burn pathophysiology in man. I. Sequential hemodynamic alterations. J Surg Res 14:64-73, 1973.
8. Johnson GS, Blalock A: Experimental shock. XII. Study of effects of hemorrhage, of trauma to muscles, of trauma to intestines, of burns and of histamine on cardiac output and on blood pressure of dogs. Arch Surg 23:855-863, 1931.
9. Turbow ME: Abdominal compression following circumferential burn: Cardiovascular responses. J Trauma 13:535-541, 1973.
10. Peterson HD, Agee RN, Andes WA, et al: Clinical Operation, Center for Treatment of Burned Soldiers, Annual Research Progress Report, US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas, 30 June 1975, pp 1-37.

can be classified as "irreversible burn shock" (11). Shoemaker et al (7) refer to this as "rapidly fatal" thermal injury and suggest that "myocardial depression" is responsible. Baxter's group also attributes the failure to achieve an adequate cardiac output to the presence of a circulating serum myocardial depressant factor (12,13). Direct measurement of left ventricular myocardial contractility has not been reported.

This study prospectively evaluated the hemodynamic profiles (cardiac output and myocardial contractility) in four severely burned patients who could not be adequately resuscitated.

METHODS AND PATIENT POPULATION

Forty-eight patients with a total body surface burn (TBSB) of greater than 30% were studied within the first 16 hours postburn. Forty-four of these have been reported previously (14). This study reports the findings in four of the patients who had to be removed from the original protocol. All had difficult crystalloid resuscitations without re-establishing an adequate cardiac output within 24 hours.

They were studied with serial hemodynamic and echocardiographic (ECHO) measurements. The methods have been previously described (14). Table 1 lists the ECHO measurements analyzed. The studies were initiated early in the postburn period (6 ± 4 hours) and continued until the therapeutic course was changed or the patient died (29 ± 13 hours postburn).

The patient characteristics are listed in Table 2. They were somewhat older (43 ± 5 years) than those in the previous report (32 ± 14 years), had extensive TBSB (80 ± 13 per cent) and received large volumes of crystalloid solution (29 ± 13 liters of Ringer's lactate/24 hours). All four had severe inhalation injury by bronchoscopy (bronchial edema and erythema, carbon particles, and

11. Dorethy JF, Treat RC: Cardiovascular function in irreversible burn shock. *Circ Shock* 5:186-187, 1978 (Abstract).

12. Baxter CR, Shires T: Physiological response to crystalloid resuscitation of severe burns. *Ann NY Acad Sci* 150:874-894, 1968.

13. Baxter CR: Fluid volume and electrolyte changes of the early postburn period. *Clin Plast Surg* 1:693-703, 1974.

14. Dorethy JF, Welch GW, Treat RC, Mason AD Jr, Pruitt BA Jr: Sequential hemodynamic alterations in severe thermal injury in the military population: Colloid-crystalloid versus crystalloid fluid resuscitation. Annual Research Progress Report, US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas, 30 September 1977, pp 120-138.

Table 1. Echocardiographic Measurements of Left Ventricular Function

1. LV end-diastolic volume (cc) = EDV
2. LV end-diastolic volume index (cc/m²) = EDVI
3. LV end-systolic volume (cc) = ESV
4. LV end-systolic volume index (cc/m²) = ESVI
5. Stroke volume (cc/bt) = SV
6. Stroke index (cc/bt/m²) = SI
7. ECHO-cardiac output (L/min) = CO
8. ECHO-cardiac index (L/min/m²) = CI
9. Ejection fraction (%) = EF
10. Mean velocity of diameter shortening (circ/sec) = V_{cf}

ulceration). All required volume ventilatory support after episodes of acute respiratory insufficiency and emergency intubation.

Table 2. Patient Characteristics

Patient	Age (yr)	TBSB (%)	Time Postburn (hr)	Ringer's Lactate (L/24 hr)	Burn to Death (hr)
A	58	61	3	20.0	26
B	45	82	9	24.5	24
C	45	83	9	26.0	18
D	23	93	2	24.8	48
Mean SD	43±15	80±13	6±4	24.8±3.9	29±13

TBSB = total body surface burn.

RESULTS

The clinical, metabolic and hemodynamic data are listed in Table 3. Three patients failed to achieve a normal cardiac index (CI) during the period of study. The composite CI was extremely low (2.7 ± 0.7 L/min/m²). In spite of this low CI, arterial pressure and urine output could be maintained with large volumes of crystalloid (approximately 2 to 3 L/hr or 10.2 ± 6 cc/kg/% TBSB). The metabolic acidosis (pH 7.26 ± 0.19) was a further reflection of inadequate circulation and was present despite large doses of sodium bicarbonate.

Table 3. Echocardiographic Hemodynamic Data in Persistent Burn Shock

Patient	CI	EDVI	ESVI	SI	EF	V _{cf}	pH
A	3.3	42±14	7±4	36±10	81±3	1.50±0.14	7.36±0.21
B	1.9	22±2	4±1	18±2	82±4	2.03±0.17	7.39*
C	2.7	38±18	13±7	23±9	67±8	1.85±0.37	7.29±0.10
D	2.9	37±1	5±3	32±2	87±8	2.36±0.44	7.03±0.10
Mean SD	2.7±0.7	35±13	8±6	27±10	78±10	1.93±0.41	7.26±0.19

See abbreviations in Table 1. Normal values: CI = 3.40 ± 0.04 L/min/m², EDVI = 59 ± 15 cc/m², ESVI = 16 ± 6 cc/m², SI = 43 ± 10 cc/bt/m², EF = 87 ± 8 , V_{cf} = 1.22 ± 0.22 circ/sec, pH = 7.38 ± 0.02 ; * only one arterial blood gas available; $p < 0.01$ compared to normal.

The CI was low initially in all four patients, and only one of these returned to normal (Fig. 1A). The CI of two patients was returning toward normal at 18 hours, but both remained acidotic (7.12 ± 0.04 , Fig 1B). The one patient with the normal CI (Patient A) also had the highest pH. He experienced acute respiratory failure at 20 hours postburn with a subsequent pH of 7.02 torr, and required colloid therapy after that period.

Echocardiographic left ventricular (LV) measurements revealed extremely low volume in systole and diastole (EDVI = 35 ± 13 cc/m² and ESVI = 8 ± 6 cc/m²). This resulted in a markedly decreased stroke index (27 ± 10 cc/bt/m², $p < 0.01$ compared to normal). These were measured simultaneously with the thermal dilution CI and were consistent with a decreased intravascular volume.

All of the patients exhibited excellent LV function. Echocardiographic ejection fraction was elevated in 3/4, and normal in the fourth (Fig. 2A). Mean velocity of diameter shortening was consistent with myocardial hypercontractility (Fig. 2B). Patient A had normal ECHO-EF and V_{cf}. The other three all exhibited hypercontractility which coincided with their extremely low CI. Patient D decreased his ejection dynamics slightly during the 6- to 12-hour period, but these returned to supranormal levels just prior to his demise (EF = 88%, V_{cf} = 2.86 circ/sec).

DISCUSSION

Persistent burn shock is rare and has an incidence of 2% to 4% in patients with large total body surface area involvement (> 50%

CARDIAC INDEX AND ARTERIAL PH DURING PERSISTENT BURN SHOCK

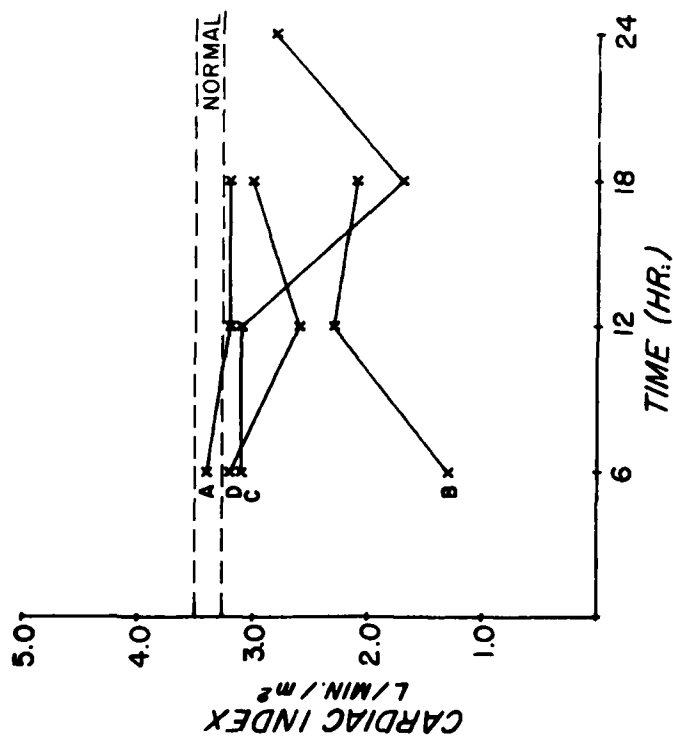


Figure 1A

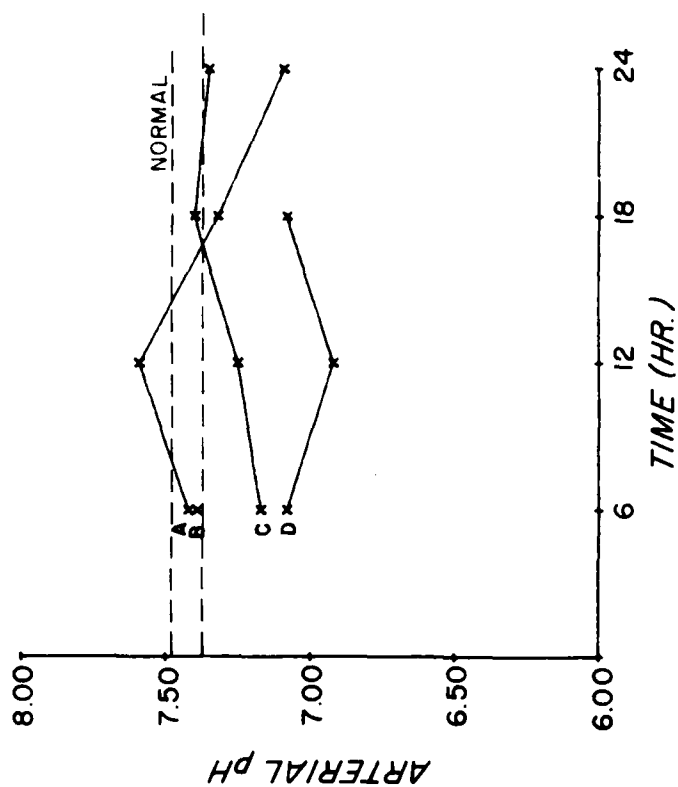


Figure 1B

ECHO LEFT VENTRICULAR FUNCTION DURING PERSISTENT BURN SHOCK

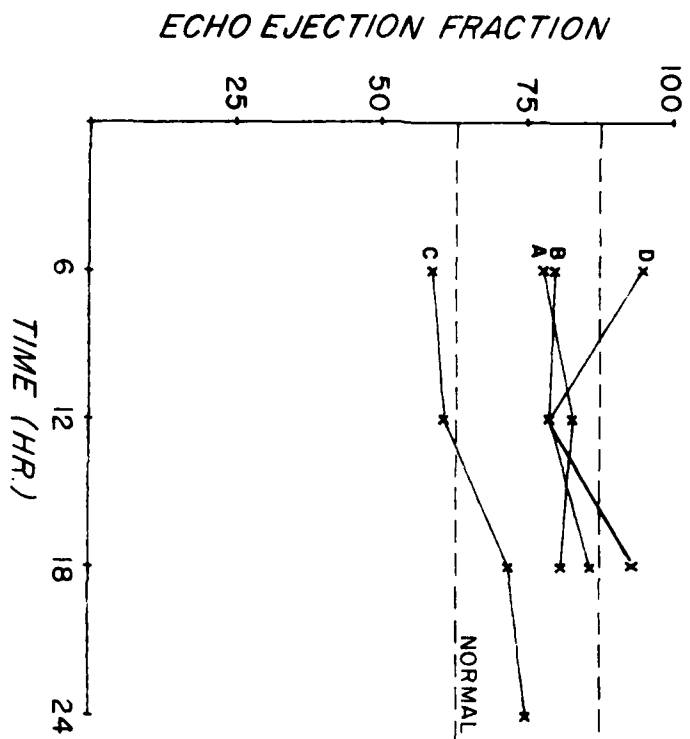


Figure 2A

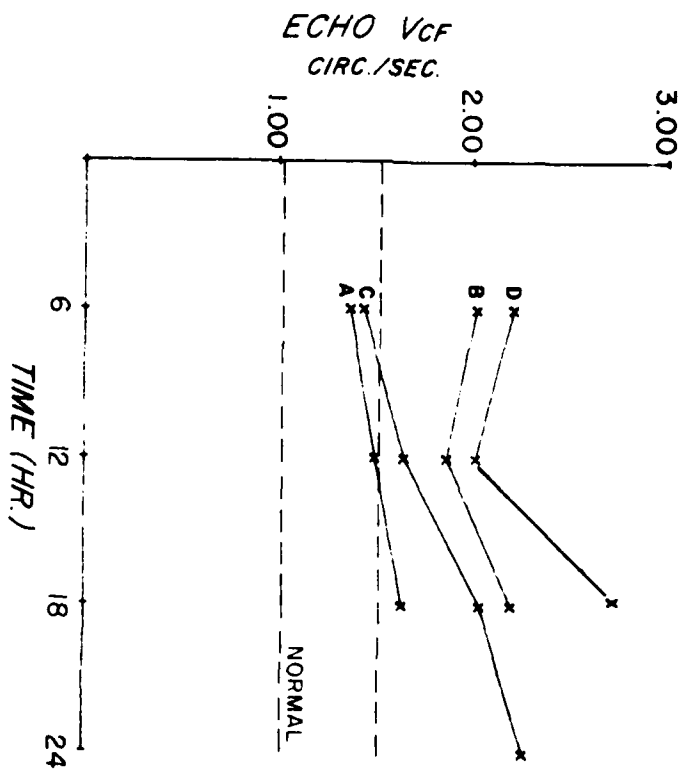


Figure 2B

TBSB). The etiology of this irreversible state is unknown, but myocardial depression has been implicated (12,13). Our previous report failed to recognize any evidence of myocardial depression in early postburn fluid resuscitation (14). However, none of those patients exhibited persistent burn shock. This study reports the findings on four patients excluded from the previous protocol.

The patients manifested burn shock by low CI, initial arterial hypotension and oliguria. They were allocated to a crystalloid volume resuscitation group, but because of the inability to re-establish adequate circulatory support, colloid infusions were necessary prior to 24 hours. All of the patients had objective evidence of inhalation injury and experienced acute respiratory insufficiency early in their postburn course. The increased volume resuscitation requirement of patients with smoke inhalation has been previously reported (13).

Therefore, these patients manifested irreversible burn shock which was rapidly fatal despite intensive therapeutic efforts. An evaluation of their CI, estimated LV filling pressure and persistent acidosis in the face of apparent adequate volume resuscitation would suggest myocardial depression. However, the direct ECHO measurements of myocardial contractility do not support this finding.

All of the patients exhibited hypercontractile LV function (increased V_{cf}) and normal to increased EF during the persistence of an inadequate CI and metabolic acidosis. On the other hand, the directly measured LV-EDV was consistent with a decrease in intravascular volume, despite more than adequate fluid replacement. At no time during the first 24 hours did any of the patients have abnormal LV function suggestive of myocardial depression.

The etiology of this inability to retain intravascular volume is unclear. An increase in pulmonary vascular resistance appears to be ruled out on the basis of normal right-sided pressure. An interstitial-intravascular oncotic disequilibrium may be present but needs to be evaluated further. Capillary permeability may be an active mechanism at injured sites but is doubtful as a generalized causative mechanism.

In summary, this study does not support the presence of myocardial depression in severely burned individuals with persistent burn shock and severe inhalation injury. They are characterized by inadequate CI, unresponsive metabolic acidosis, early acute respiratory failure, decreased LV intravascular volume, and excellent LV ejection dynamics. These patients represent a distinct subgroup from those previously reported, although their LV hemodynamic profiles are similar.

PRESENTATIONS

Dorethy JF: Central hemodynamics of burn shock: Crystalloid vs colloid resuscitation. Surgery Conference, University of Texas Health Science Center at San Antonio, San Antonio, Texas, 3 February 1978.

Dorethy JF: Evaluation of left ventricular function during early postburn resuscitation: Lack of evidence for a clinical myocardial depressant factor. Annual Meeting, American Burn Association, Birmingham, Alabama, 1 April 1978.

Dorethy JF: Cardiovascular function in irreversible burn shock. First Annual Conference on Shock, Airlie, Virginia, 1 June 1978.

Dorethy JF: The efficacy of colloid-crystalloid resuscitation fluid in the earlier re-establishment of an adequate hemodynamic state in postburn "shock." Fifth International Congress on Burn Injuries, Stockholm, Sweden, 20 June 1978.

PUBLICATIONS

Dorethy JF, Treat RC: Cardiovascular function in irreversible burn shock. Circ Shock 5:186-187, 1978 (Abstract).

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: THE HEMODYNAMIC RESPONSE TO THERMAL INJURY IN BURNED
SOLDIERS -- II. THE EFFECTS OF INTRATHORACIC PRESSURE
CHANGES ON LEFT VENTRICULAR FUNCTION DURING CONTINUOUS
POSITIVE PRESSURE VENTILATION IN THE THERMALLY INJURED
MILITARY POPULATION

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

James F. Dorethy, M.D., Lieutenant Colonel, MC
Victor Lam, M.D., Major, MC

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ABSTRACT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: THE HEMODYNAMIC RESPONSE TO THERMAL INJURY IN BURNED SOLDIERS -- II. THE EFFECTS OF INTRATHORACIC PRESSURE CHANGES ON LEFT VENTRICULAR FUNCTION DURING CONTINUOUS POSITIVE PRESSURE VENTILATION IN THE THERMALLY INJURED MILITARY POPULATION

US Army Institute of Surgical Research Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

Investigators: James F. Dorethy, M.D., Lieutenant Colonel, MC
Victor Lam, M.C., Major, MC

This study was designed to evaluate the intrathoracic pressure-volume relationships in patients with respiratory failure (RF) treated with continuous positive pressure ventilation (CPPV). The study was divided into three phases: (1) definition of hemodynamic profiles in acute RF as a complication of thermal injury, (2) left ventricular (LV) ejection dynamics and end-diastolic volume (EDV) during treatment of RF with CPPV, (3) right, left and intrathoracic pressure-volume relationships during CPPV. The first two phases have been completed. Acute RF postburn occurs as an early (< 60 hr postburn) or late complication (6 \pm 2 days). Those with late RF could be subdivided into pure pulmonary failure; LV failure without a pulmonary component, and a mixed group with both a cardiovascular and a pulmonary component. The latter two groups require invasive monitoring during treatment. Continuous positive pressure ventilation of 20 cm H₂O resulted in a 20% decrease in cardiac output in 40% of the patients. This was accompanied by a simultaneous decrease in LV-EDV. There was no deterioration in LV ejection fraction and/or mean velocity of diameter shortening. Therefore, the primary etiology of the decreased cardiac output was not myocardial depression but a change in LV filling volume. The actual net pressure-volume changes are being evaluated in a continuation of this study.

Burn injury
Cardiac output
Respiratory failure
Echocardiography
Cardiovascular hemodynamics

THE HEMODYNAMIC RESPONSE TO THERMAL INJURY IN BURNED SOLDIERS. II.
THE EFFECTS OF INTRATHORACIC PRESSURE CHANGES ON LEFT VENTRICULAR
FUNCTION DURING CONTINUOUS POSITIVE PRESSURE VENTILATION IN THE
THERMALLY INJURED MILITARY POPULATION

Inhalation injury sustained during thermal injury is associated with varying degrees of respiratory insufficiency. Continuous positive pressure ventilation (CPPV) is widely used in the treatment of this condition (1). The effects of such therapy on cardiopulmonary performance as well as the definition of "optimal CPPV" are controversial (2-9). Optimal CPPV should allow for the improvement in PaO_2 without deterioration in cardiovascular performance and minimum barotrauma (7,8,10,11). The accurate assessment and manipulation of

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cardiopulmonary function during CPPV is difficult, because of the complex interaction of pleural, alveolar, and vascular pressures (12-20).

Experimental studies with direct measurement of ventricular filling pressure have given some insight into the significance of intrathoracic pressure (ITP) reflection (21-23). Pontoppidan's group concluded in dogs that a reduction in ventricular filling pressures, as referred to pleural pressure, is the cause of the decreased cardiac

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12. Lozman J, Powers SR Jr, Older T, Dutton RE, Roy RJ, English M, Marco D, Eckert C: Correlation of pulmonary wedge and left atrial pressures: A study in the patient receiving positive end expiratory pressure ventilation. *Arch Surg* 109:270-277, 1974.
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ANNUAL RESEARCH PROGRESS REPORT. 1 OCTOBER 1977-30 SEPTEMBER 19--ETC(U)

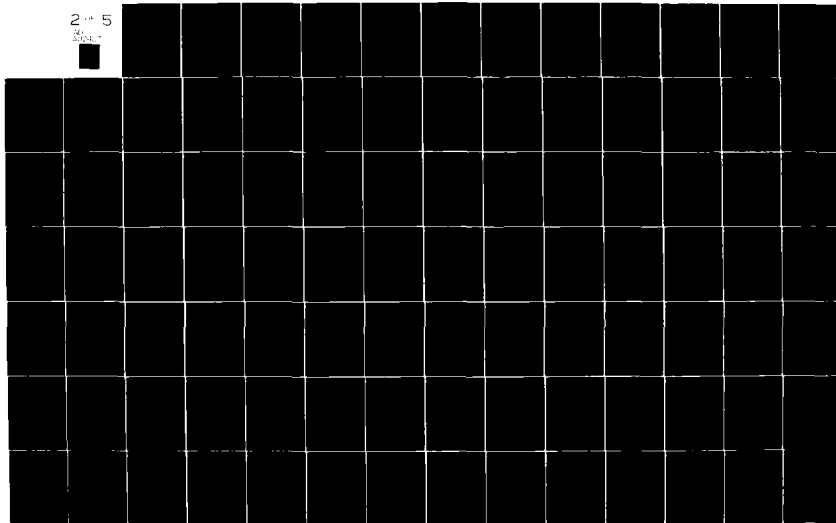
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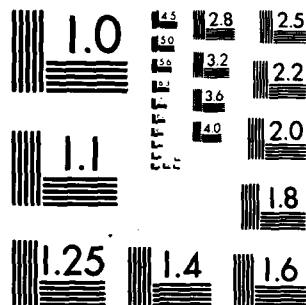
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index associated with levels of CPPV greater than 10 cm H₂O (21). They also showed that this change was sustained for prolonged periods of time, that increases in blood volume blunted the response, and that the hemodynamic response of hypervolemia was manifested when CPPV was discontinued.

Pouleur et al, in attempting to define a critical airway pressure, noted that the transpulmonary pressure resulted in a linear augmentation of pulmonary pressures despite concomitant decreases in flow (22). The linear relationship was most significant in changes in pulmonary artery end-diastolic pressure. They suggested that the knowledge of this pressure makes possible the adaption of CPPV to its maximal value: before the occurrence of significant hemodynamic effects. Other evidence suggests that the central venous pressure may be more representative of the reflection of ITP (transpulmonary pressure) changes (24,25). On the other hand, left atrial pressure is the least affected by ITP changes, and its passive reflection to the pulmonary capillary wedge (PCW) is often inaccurate (12,26). This may lead to erroneous decisions concerning left ventricular (LV) function and the adequacy of volume replacement (12,27). Experimental animal studies have noted no evidence of intrinsic LV dysfunction with CPPV (21,23).

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However, some clinical studies have implied that LV dysfunction is often a consequence of CPPV greater than 15 cm H₂O (12,28), and that PCW did not predict this impending failure. In addition, this pressure did not accurately reflect left atrial pressure (12,27). Peters' group, studying post-traumatic pulmonary insufficiency, concluded that when an increased PCW accompanied a decreased cardiac output (CO), it most likely represented a reflection of ITP and not left atrial pressure (27). This is known as the Starling resistor effect (29-31). This hypothesis suggests that increased ITP restricts flow through the pulmonary circulation with a resultant decrease in CO. The decrease in venous return noted in earlier studies would be a consequence rather than a cause of hemodynamic deterioration noted with CPPV. Another result of transmitted ITP to pulmonary vascular structures would be a net positive increase in impedance to right ventricular ejection (27). Similar to animal studies, most clinical work has concluded that an increase in PCW is an ominous sign and cannot be interpreted as volume overload and/or LV failure (12,27,32). However, Kirby et al, in patients treated with up to 18 torr (25 cm H₂O) of CPPV found no signs of decreases in CO and actually recorded an increase in 75% (2). Other studies have found significant decreases

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in CO with increases in CPPV over 10 cm H₂O (3,6,29,33-35).

This study was designed to evaluate acute respiratory failure (ARF) as a complication of thermal injury and to study the LV hemodynamic profiles associated with ARF and its treatment with CPPV. The results obtained during CPPV are the subject of previous reports (36-37). This communication reports the clinical and hemodynamic findings in ARF as an early and late complication of thermal injury.

METHODS AND PATIENT POPULATION

Any patient requiring CPPV and pulmonary artery pressure monitoring was considered for inclusion into the study. Excluded were patients with hemodynamic instability, bullous emphysema, or CNS trauma. The criterion for intubation and institution of CPPV was ARF manifested by refractory hypoxemia. This was quantified as the inability to maintain a PaO₂ of > 60 torr with a 50% FIO₂. The patients were sedated or paralyzed as necessary. Volume-cycled ventilators were utilized via an appropriate endotracheal tube. Initial settings attempted to establish a PaO₂ > 60 torr and a PaCO₂ of < 40 torr.

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Pressure monitoring was performed by a percutaneous Swan-Ganz CO thermal dilution catheter and a special arterial catheter passed retrograde to the central aorta. It then was advanced into the LV cavity with continuous ECG and pressure monitoring. Catheter positions were confirmed with pressure recordings and portable chest x-ray. Pulmonary airway pressure was measured with a #4 Millar micromanometer inserted into the upper airway through a special port in the endotracheal tube connections. A #6 F Millar micromanometer was inserted into the esophagus at about 20-25 cm in a specially made plastic sheath. Pulmonary volume and flow rates were measured with a pneumotachograph and processed with a Validyne carrier modulator and integrator.

Cardiopulmonary hemodynamics and pulmonary volume-flow measurements as shown in Table 1 were obtained at zero (0) CPPV. These were repeated at each subsequent phase. Pressure profiles as listed in Table 2 were also measured at each phase. CPPV was added and confirmed by the measurement of airway pressure (AirP). Initially, CPPV was 7 torr (10 cm H₂O). These measurements were then repeated at 11.5 torr (15 cm H₂O) and 14.5 torr (20 cm H₂O). The equilibration time was 20 minutes at each setting. Following completion of the above, CPPV was discontinued and a second set of baseline studies obtained. Echocardiographic measurements of LV internal dimensions were taken at each phase as previously described (38).

RESULTS

To date, only preliminary data have been obtained in four patients to examine the limitations of the methodology. A previous report summarized the pressure-volume data in 11 patients during the use of 20 cm H₂O CPPV (36). The data from 29 patients with ARF are presently being analyzed and will be reported at a later date.

DISCUSSION

The utilization of CPPV in the treatment of acute respiratory distress syndrome following inhalation injury creates complex cardiopulmonary interactions. The necessity of correctly assessing volume adequacy in thermally injured patients requires an understanding of

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Table 1

Cardiovascular Pressures, Intrathoracic Pressures,
and Pulmonary Flow-Volume Measurements

1. Cardiovascular Measurements

A. Right side pressures (P)

Right atrial pressure (RAP and CVP)
Pulmonary pressure (PuPs, PuPed, and \bar{P}_u)
Pulmonary capillary wedge (PCW)

B. Left side pressures

Aortic pressure (AoP)
Left ventricle systolic (LVsP)
Left ventricle end-diastolic (LVedP)

2. Intrathoracic Pressures

Airway (AirP)
Esophagus (EP)

3. Flow-Volume Measurements

A. Cardiac output (CO)

Fick (CO_F)
Thermal dilution (CO_{TD})

B. Ventilatory flow-volume

Tidal volume (TV)
Functional residual capacity (FRC)
Flow rate and profile

4. Arterial Blood Samples

Arterial O_2 tension (PaO_2)
Arterial CO_2 tension ($PaCO_2$)
Arterial saturation ($Sat_a\%$)
Venous saturation ($Sat_v\%$)

Table 2

Pressure Profile Sets

-
1. LVsP, LVedP, RAP*, Pup[†], AoP[†], EP, and AirP (mm Hg);
AoP[†] measured with continuous pullback.
 2. LVsP, LVedP, RAP*, PCW*, AoP[†], EP, and AirP (mm Hg);
AoP[†] measured with continuous pullback.
-

- * Phasic pressures ("a", "v" waves) with electrical mean.
- † Phasic pressures (systolic and diastolic) with electrical mean.

these interactions. This study was initiated to analyze the relationship of LV function following the effects of CPPV on CO and pleural, alveolar and vascular pressures by simultaneous direct measurement of cardiopulmonary parameters. The study population at the present time is too small for meaningful statistical evaluation. The study is to be continued.

PRESENTATIONS

Dorethy JF: The effect of positive end expiratory pressure (PEEP) on left ventricular (LV) function in patients with respiratory failure. American College of Chest Physicians 43rd Annual Scientific Assembly, Las Vegas, Nevada, 3 November 1977.

Dorethy JF: Left ventricular volume and ejection indices during positive end expiratory pressure. American Heart Association 50th Annual Scientific Sessions, Miami Beach, Florida, 29 November 1977.

Dorethy JF: The relationship of left ventricular performance and decreased cardiac output during positive end expiratory pressure. Association of Army Cardiology 7th Annual Session, Washington, DC, 6 May 1978.

PUBLICATIONS

Dorethy JF, Lam V: Left ventricular volume and ejection indices during positive end expiratory pressure. Circulation 56:138 (Suppl III), 1977 (Abstract).

ANNUAL PROGRESS REPORT

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SHOCK WITH DOPAMINE

US ARMY INSTITUTE OF SURGICAL RESEARCH
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Richard C. Treat, M.D., Major, MC

Reports Control Symbol MEDDH-288(R1)

Left ventricular (LV) hemodynamic profiles during septic shock are not well defined. This study evaluated LV ejection dynamics and their relationship to cardiac output (CO, L/min) in 10 thermally injured patients with septic shock. Myocardial performance was measured directly by serial echocardiographic LV ejection fraction and mean velocity of diameter shortening; CO was measured by thermal dilution. Three distinct subgroups were recognized. (1) Hyperdynamic septic shock (n = 4): These patients exhibited high CO with excellent ejection fraction and mean velocity of diameter shortening. Dopamine treatment had no significant effect on their CO or LV function. However, it was useful in re-establishing and maintaining an adequate urine output and arterial pressure in this group. (2) Low output septic shock (n = 4): These patients initially had decreased CO and variable LV function (two normal and two abnormal). In this group, early dopamine therapy increased the CO and the LV ejection indices. (3) Myocardial failure septic shock: Two patients with autopsy proven staphylococcal myocardial abscesses had hyperdynamic LV function which became abruptly abnormal. Dopamine had no effect on their rapidly fatal clinical course. Conclusions: The primary cardiovascular response during septic shock can be classified as hyperdynamic, low output, or myocardial failure based upon the relationship of LV ejection dynamics to CO.

Septic shock
Left ventricular function
Dopamine
Echocardiography

ECHOCARDIOGRAPHIC LEFT VENTRICULAR VOLUME AND EJECTION INDICES DURING THE TREATMENT OF SEPTIC SHOCK WITH DOPAMINE

Severe bacteremia and subsequent cardiopulmonary collapse continue to be a common cause of death in the post-resuscitated severely burned patient (4-10 days postburn). The incidence of "septic shock" in the face of persistent bacteremia is suggested to be 25% to 41% (1,2). There is a less frequent incidence of septic shock in gram-positive bacteremias, while the gram-negative organisms show an appreciable species difference in the frequency of occurrence (2). Although the overall incidence of septic shock in a thermally injured population is unknown, it appears to be high. The cardiovascular manifestations of bacteremia are divided into a "pyrogenic" and a "septic" phase. Pulmonary dysfunction as a primary manifestation of septic shock has recently received a great deal of attention (3-4). The hemodynamic and metabolic consequences in each phase are complicated and multifactorial (5-10). The most important aspect is a dynamic relationship between left ventricular output and peripheral vasomotor activity. This starts as a "hyperdynamic" state which is replaced in time by unresponsive hypotension and anuria (9-11).

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Left ventricular dysfunction has been postulated (10,12) and is used to defend the utilization of inotropic agents (i.e., digoxin and various catecholamines). Dopamine (a precursor of norepinephrine) is an agent commonly used in attempts to restore adequate tissue perfusion when volume expansion is unsuccessful. "Intrinsic myocardial contractility (i.e., left ventricular ejection fraction and mean velocity of diameter shortening) has not been evaluated in the different phases of bacteremia nor during its treatment.

The effect of dopamine on cardiovascular function is related to infusion dosage and varies in individual patients (13-15). The usefulness in various shock syndromes has been extensively evaluated (10, 16-19). However, it is least effective in well documented septic shock (12,16). The usefulness of dopamine in the pyrogenic phase of bacteremia is unknown. The dopaminergic effect on the heart has been postulated to be coronary vasodilation (20-22). Its primary difference

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from other catecholamines is its ability selectively to vasodilate renal and mesenteric vessels (23,24) The purpose of this study was to define a hemodynamic profile of septic shock as it relates to myocardial contractility and to evaluate the usefulness of dopamine treatment.

METHODS AND PATIENT POPULATION

Ten thermally injured patients were evaluated at the clinical onset of sepsis (defined by two consecutive positive blood cultures) and after the development of septic shock (Table 1). Six of the 10 had persistent hypotension (< 100 mm Hg) which required dopamine after initial fluid challenges failed to maintain an adequate arterial pressure. All of the patients exhibited hypotension sometime during the study course, and all but one were eventually placed on dopamine support. All received broad-spectrum antibiotic coverage. Six of the patients required mechanical ventilation very early in the study, and all but one required respiratory support sometime during their course.

Table 1. Clinical Criteria for the Diagnosis of Septic Shock

-
1. An absolute criterion was the presence of bacterial sepsis defined as two consecutive positive venous blood cultures.
 2. Hypotensive episode and/or persistent hypotension (systolic < 100 mm Hg).
 3. In addition, one of the following was present:
 - A. Mental confusion, obtundation, seizures, and/or coma.
 - B. Marked urinary output changes: oliguria (< 20 cc/hr) or anuria.
 - C. Unexplained respiratory failure (< 60 PaO₂ on 50% FIO₂).
-

The hemodynamic aspects of septic shock were evaluated by serial

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echocardiography as previously described (25). Table 2 lists the echocardiographic measurements utilized to evaluate myocardial contractility. These were performed at various time intervals depending upon the clinical course. At least one study was performed prior to the patient being placed on dopamine. Statistical analysis was performed utilizing the unpaired Student's t-test.

Table 2. Echocardiographic Measurements of Left Ventricular Function

1. LV end-diastolic volume (cc) = EDV
2. LV end-diastolic volume index (cc/m ²) = EDVI
3. LV end-systolic volume (cc) = ESV
4. LV end-systolic volume index (cc/m ²) = ESVI
5. Stroke volume (cc/bt) = SV
6. Stroke index (cc/bt/m ²) = SI
7. ECHO-cardiac output (L/min) = CO
8. ECHO-cardiac index (L/min/m ²) = CI
9. Ejection fraction (%) = EF
10. Mean velocity of diameter shortening (circ/sec) = V _{cf}

RESULTS

Patient characteristics are listed in Table 3. The study population was young (24 ± 8 years) with large total body surface burns

Table 3. Patient Characteristics

Number of patients	10
Age (years)	24 ± 8
Total body surface burn (%)	58 ± 6
Cultures	10/10
Gram-negative	5/10
Gram-positive	3/10
Mixed	2/10
Time of sepsis postburn (days)	$7 \pm 5^*$
Inhalation injury	4/10
Respiratory failure	9/10
Mortality	8/10
Autopsy	6/8

* Two of the 10 patients exhibited septic shock more than 30 days postburn.

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(58 ± 6 percent). The type of initial fluid resuscitation did not influence the incidence of septic shock. All patients had central lines for varying periods of time postburn, and this was not different in the individual patients.

All the patients had positive blood cultures; five had only gram-negative organisms, three gram-positive, and two mixed organisms recovered. Eight of the 10 became septic within 7 ± 5 days postburn. Two patients developed sepsis and shock after being in the hospital for extended periods (32 and 72 days respectively). The bacterial organisms thought to be responsible for the shock are listed in Table 4. No attempt was made to define a particularly incriminating entrance focus, although six patients had positive wound invasion (Table 5).

Table 4. Bacterial Organisms Responsible for Septic Shock in 10 Patients

Gram-negative	
Pseudomonas aeruginosa	5*
Klebsiella	1
Gram-positive	
Staphylococcus	3
Mixed	
Staph, Pseudomonas, and Klebsiella	2

* One patient had both Pseudomonas and Klebsiella recovered from blood cultures.

Respiratory failure was a prominent clinical feature occurring in all but one of the patients. An initial diagnosis of inhalation injury was made in four of the nine patients. The interaction of bacteremic-induced respiratory failure superimposed on inhalation injury is complex and not well understood.

Eight of the 10 patients died, and autopsies were obtained in six. The postmortem findings are typical of those found in severe septicemia (Table 5). The systems most prominently involved were pulmonary (6/8) and renal (4/8). None of the patients had atherosclerotic heart disease or chronic valvular disease.

Utilizing the left ventricular hemodynamic profile, the patients could be divided into three subcategories (Table 6). Table 7 lists the hemodynamic profiles prior to clinical sepsis. The hyperdynamic septic shock (HSS) was distinguished by elevated initial cardiac

Table 5. Autopsy Findings in Six Patients with Septic Shock

Cardiovascular	2
Myocardial abscesses	2
Myocardial infarct	1
Acute bacterial endocarditis	1
Pericardial effusion	1
Pulmonary	6
Hematogenous pneumonia	4
Septic thromboembolism	1
Multifocal hemorrhage	6
Pleural effusion	3
Renal	4
Central nervous system	2
Adrenal hemorrhage	2
Burn wound invasion	6

Table 6. Subclassification of Septic Shock by Left Ventricular Hemodynamic Profile

1. Hyperdynamic septic shock (HSS).

n = 4. Patients with elevated CO and normal LV ejection dynamics (ECHO-EF and V_{cf}). Dopamine of no inotropic assistance, but useful in increasing urinary output and elevating blood pressure.

2. Low output septic shock (LOSS).

n = 4. Patients with low CO and variable ECHO LV function. Dopamine initially helpful in improving both LV function and CO, but clinical course unchanged.

3. Myocardial abscess septic shock (MASS).

n = 2. Patients with abrupt deterioration of ECHO LV function and rapidly fatal clinical course. Dopamine was ineffective.

output (11.8 ± 2.3) and excellent left ventricular ejection dynamics ($EF = 78 \pm 4$, $V_{cf} = 1.68 \pm 0.48$). The patients designated as low output septic shock (LOSS) initially had a lower output (4.7 ± 1.2 L/min) and variable left ventricular function (two normal and two abnormal). The two patients with postmortem myocardial abscesses had a distinctly different course when compared to the HSS or LOSS. They exhibited an abrupt deterioration of previously excellent left ventricular function and cardiac output ($CO = 9.6 \pm 2.1$ versus 2.3 ± 1.3 , $EF = 84 \pm 6$ versus 40 ± 6 , and $V_{cf} = 2.02 \pm 0.43$ circ/sec versus 0.90 ± 0.10). They had a rapid downhill course, despite adequate therapy.

Table 7. Left Ventricular Function at Clinical Onset of Sepsis

Group	n	Cardiac Output (L/min)	ECHO-EF (%)	ECHO- V_{cf} (circ/sec)
HSS	4	$11.8 \pm 2.3^*$	78 ± 4	$1.68 \pm 0.48^*$
LOSS	4	4.7 ± 1.2	74 ± 6 52 ± 4	1.48 ± 0.26 1.00 ± 0.12
MASS	2	9.6 ± 2.1	$84 \pm 6^*$	$2.02 \pm 0.43^*$

Values = mean \pm 1 SD; n = number of patients; EF = ejection fraction, V_{cf} = mean velocity of diameter shortening; normal: $CO = 6.8 \pm 1.2$ (L/min); $EF = 74 \pm 6$ (%), $V_{cf} = 1.22 \pm 0.22$ (circ/sec); * $p < 0.05$ compared to normal.

Dopamine treatment successfully altered the cardiac output and ejection dynamics in LOSS but did not significantly change their fatal outcome (Table 8). Dopamine therapy did not alter the hemodynamic profiles in the three HSS patients who received that agent.

All of the patients with LOSS had a fatal outcome regardless of their initial or dopamine treated left ventricular ejection dynamics. All had gram-negative organisms which were responsible for their shock. The patients who lived were in the HSS category ($n = 2$), and both had staphylococcal-induced septic shock. The other two HSS patients had gram-negative shock. Therefore, all of the patients with gram-negative infection died, but not all had LOSS. In both patients with myocardial abscess septic shock (MASS), the responsible organism for myocardial failure was *Staphylococcus*, while one of the two also had positive *Pseudomonas* cultures.

DISCUSSION

The hemodynamic response to septic shock has been defined as hyper- or hypodynamic based entirely on indirectly measured indices

Table 8. Left Ventricular Function during Treatment of Septic Shock with Dopamine

Group	n	Cardiac Output (L/min)	ECHO-EF (%)	ECHO-V _{cf} (circ/sec)
HSS	3	10.7 ± 1.9	76 ± 2	1.76 ± 0.52
LOSS	4	6.5 ± 1.2*	76 ± 4 62 ± 6	1.60 ± 0.40 1.22 ± 0.12
MASS	2	2.3 ± 1.3*	40 ± 6*	0.90 ± 0.10*

Values = mean ± 1 SD: see Table 7 for abbreviations and normal values; * p < 0.05 compared to pretreatment.

of left ventricular performance, i.e., cardiac output, pulmonary capillary wedge pressure, etc. (6,7,10,26-29). Various etiologies for the decreased cardiac output noted with hypodynamic septic shock have been the subject of wide speculation (7,26,29). In this regard, experimental animal studies are not directly comparable to human studies (30). This study attempts to clarify the classification of hemodynamic response to sepsis by utilizing direct measurements of myocardial contractility and comparing it to the cardiac output response.

The findings of the present study confirm the presence of a high and low output state in patients with well documented septic shock. In addition, a third category was recognized in two patients with documented myocardial abscesses. The serial ECHO LV function studies did not make it readily apparent whether the HSS and LOSS states were time dependent and/or related to the severity of bacteremia. The only obvious deterioration of LV function in HSS occurred in the immediate preterminal period. These patients exhibited no evidence of

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myocardial depression prior to that time. Those patients with hyperdynamic septic shock (HSS) had excellent left ventricular function associated with an increased cardiac output. The only survivors were in this group, both with gram-positive infections (Staphylococcus). However, the hyperdynamic state was not limited to gram-positive infections. In HSS, the addition of dopamine did not improve the overall excellent left ventricular function.

Those exhibiting low output septic shock (LOSS) had variable left ventricular function. Two of the patients had well preserved function and no evidence of myocardial depression. The two with abnormal function had similar clinical courses and causative organisms and could not otherwise be distinguished from those with normal function. All four patients with LOSS responded to dopamine therapy initially but failed to sustain the improvement.

The most dramatic hemodynamic response occurred in two patients with autopsy documented myocardial abscesses. They had an abrupt deterioration of the left ventricular performance, followed by a rapidly fatal course. Obvious direct myocardial depression was present. They exhibited little or no response to dopamine therapy.

All of the patients with LOSS had gram-negative organisms as causative agents. Also, all of the patients infected with gram-negative organisms died, but two of them were initially classified as HSS. Therefore, the low output state is not unique to these organisms but is associated with a high mortality.

In summary, the data presented suggest that the hemodynamic status of a patient in septic shock is different and may depend upon time of onset or severity of bacteremia. Inotropic effectiveness depends upon the severity of response to sepsis, i.e., HSS versus LOSS. Hyperdynamic septic shock occurred as frequently as LOSS and was caused by both gram-positive and gram-negative organisms. The mortality was lower in individuals with gram-positive sepsis. Left ventricular function was variable in LOSS, with myocardial depression present in some patients. Direct myocardial involvement was obviously responsible for MASS.

PRESENTATIONS

Dorethy JF: The hemodynamic classification of septic shock by left ventricular ejection dynamics and cardiac output. First Annual Conference on Shock, Airlie, Virginia, 3 June 1978.

PUBLICATIONS

Dorethy JF: The hemodynamic classification of septic shock by left ventricular ejection dynamics and cardiac output. Circ Shock 5:217-218, 1978 (Abstract).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				DA OG 6973		78 10 01		78 10 01	
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33. TECHNICAL OBJECTIVE, 34. APPROACH, 35. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.) 23. (U) To document and evaluate the gastrointestinal changes in burned soldiers. To study the role of pepsinogen in the etiology of Curling's ulcer and the effectiveness of cimetidine in ulcer prophylaxis. To assess the role of infection on stress ulcers and changes in small bowel morphology and function. To evaluate etiologies of stress ulcers. 24. (U) Esophageal, gastric and duodenal mucosal lesions will be verified endoscopically and with biopsies in burn patients. Mucosal lesions will be related to serum pepsinogen levels and the effect of cimetidine determined. Small bowel structure and function will be evaluated and the effect of bacterial flora assessed. A laboratory animal model of stress ulcer will be developed. 25. (U) 7710 - 7809 Both the pepsinogen and cimetidine studies have been completed. The pepsinogen study showed no meaningful correlation between serum pepsinogen levels and endoscopic findings. The cimetidine study showed cimetidine to be equally as effective as antacids in the prevention of stress-induced gastroduodenal complications. Cimetidine's lack of major side effects and ease of administration make it an attractive alternative to antacid therapy. Animal studies have shown that cimetidine confers a protective effect on gastric mucosal blood flow in shocked animals. Animal studies investigating the role of the gastrointestinal system in host response to thermal injury and associated infection have been discontinued. The effect of cimetidine of gastric mucosal blood flow has been evaluated and the agent found to be protective. Studies evaluating the anti-edema effect of cimetidine are in progress.									

DD FORM 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORM 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

FINAL REPORT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: GASTROINTESTINAL ALTERATIONS AND COMPLICATIONS IN BURNED
TROOPS -- SERUM PEPSINOGEN LEVELS IN THE THERMALLY INJURED
SOLDIER: A POSSIBLE PREDICTOR OF GASTRODUODENAL DISEASE

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

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(Professor of Medicine, UCLA)

Reports Control Symbol MEDDH-288(R1)

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ABSTRACT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: GASTROINTESTINAL ALTERATIONS AND COMPLICATIONS IN BURNED TROOPS -- SERUM PEPSINOGEN LEVELS IN THE THERMALLY INJURED SOLDIER: A POSSIBLE PREDICTOR OF GASTRODUODENAL DISEASE

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

Investigators: Hugh P. McElwee, M.D., Major, MC
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Serum pepsinogen levels (PG-I) have been shown to reflect the secretory activity and morphologic status of gastric mucosa in various disease entities. However, their potential value in predicting stress ulceration has not been defined. The purpose of this study was to evaluate PG-I as an indicator of endoscopically recognized gastric mucosal change following thermal injury.

Serial upper gastrointestinal endoscopies (n = 39) were performed on 14 patients with greater than 50% mean total body surface burn. All patients were being treated with anti-ulcer therapy, and no clinically significant gastrointestinal hemorrhage was encountered. Seven patients (Group A) had normal or mild (erythema) endoscopic changes throughout the 10-day period of observation. Seven other patients (Group B) had moderate or severe endoscopic changes (erosions and/or ulcerations) at least once during a similar period of observation. Endoscopic findings were verified with random photographs and biopsies. Simultaneously obtained serum samples were analyzed for PG-I utilizing a competitive binding, double antibody, radioimmunoassay.

Table 1. Serum Pepsinogen Levels

	Admission	Daily
Group A	65 \pm 16	71 \pm 8
Group B	44 \pm 7	92 \pm 37

Values = mean \pm 1 SEM; PG-I units = ng/ml

Admission PG-I was not predictive of subsequent endoscopic findings (Group A versus Group B, $P < 0.3$). Also, no meaningful relationship was noted between daily PG-I and endoscopic mucosal appearance (Group A versus Group B, $P < 0.3$). In addition, PG-I on the day of endoscopy did not correlate with endoscopic findings ($P < 0.4$). We conclude that serum PG-I does not correlate with distinct endoscopic groups following thermal injury. These findings are consistent with reports of unaltered acid secretion and normal serum gastrin levels in this patient population.

Based on the above findings, this study has been discontinued.

Burn injury
Curling's ulcer
Gastritis
Pepsinogen

FINAL REPORT

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REPORT TITLE: GASTROINTESTINAL ALTERATIONS AND COMPLICATIONS IN
BURNED TROOPS -- THE EVALUATION OF CIMETIDINE, A NEW
H₂-RECEPTOR ANTAGONIST, IN THE PREVENTION OF STRESS
ULCERATION FOLLOWING THERMAL INJURY

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This prospective, randomized, double-blind, endoscopic study compared the efficacy of cimetidine (13 patients) to that of antacids (14 patients) in the prevention of stress ulceration following severe thermal injury. Both treatment modalities were equally effective in the prevention of acute gastroduodenal disease and its associated complications. Cimetidine and antacids produced a near elimination of duodenal disease and a marked reduction in the severity of gastric disease when compared to untreated historical controls. Lack of major side effects and ease of administration make cimetidine an attractive alternative to antacid therapy in the prophylaxis of stress-induced gastroduodenal disease in the thermally injured patient.

Burn injury
Curling's ulceration
Cimetidine

GASTROINTESTINAL ALTERATIONS AND COMPLICATIONS IN BURNED TROOPS --
THE EVALUATION OF CIMETIDINE, A NEW H₂-RECEPTOR ANTAGONIST, IN THE
PREVENTION OF STRESS ULCERATION FOLLOWING THERMAL INJURY

Acute gastroduodenal mucosal disease occurs in 80% of untreated patients following significant thermal injury and may progress to life-threatening gastric hemorrhage or perforation (1). The frequent administration of antacids has dramatically decreased these complications of stress ulceration and has become essential preventative therapy (2). Although antacids are known to have numerous potential side effects (3,4), these have not been well documented in the thermally injured patient. The effect of antacids on the endoscopically defined natural history of stress ulceration has not been evaluated.

An alternative drug in the preventative treatment of stress-induced gastroduodenal lesions in the thermally injured patient is cimetidine, an H₂-receptor antagonist (5). Its prophylactic efficacy has been reported in animal studies (6,7) and in critically ill patients (8). Its specific physiologic action, ease of administration, and lack of major side effects (9) make it an attractive alternative to antacid therapy. This prospective, randomized, double-blind study evaluated the effects of cimetidine and antacids on the natural history and prevention of stress ulceration following thermal injury.

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2. McAlhany JC Jr, Czaja AJ, Pruitt BA Jr: Antacid control of complications from acute gastroduodenal disease after burns. *J Trauma* 16:645-649, 1976.
3. Piper DW: Antacid and anticholinergic drug therapy. *Clin Gastroenterol* 2:361-377, 1973.
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MATERIALS AND METHODS

Patient Population

All patients age 18 years or older, with greater than a 30% total body surface burn area, were considered for inclusion in the study. Patients admitted within 48 hours after burn injury and who had not been treated with antacids were accepted as study candidates. Exclusion criteria included head trauma, an antecedent history of gastrointestinal hemorrhage or ulcer disease, or pregnancy. Informed written consent was obtained from all patients after careful explanation of the risks of repeated endoscopy and the use of experimental drugs.

Study Design

Patients qualifying for the study were assigned medication numbers in sequential order. These numbers were randomized to match with two forms of treatment. Group A received cimetidine as well as a placebo simulating antacid in color and appearance but without meaningful buffering capacity. Group B received an antacid (Mylanta^R-II, Stuart; buffering capacity of 4.1 mEq per milliliter) and a neutral placebo simulating cimetidine. The code which identified the medication was known only to the Research Department of Smith Kline & French which provided the medication and prepared all the therapeutic and placebo agents. Provisions were made to break the code when deemed necessary for individual patient problems.

The antacid or placebo antacid was administered at a rate of 30 milliliters every two hours. Gastric aspirates were checked for volume and pH at hourly intervals in those patients with nasogastric tubes in place. When the pH of the gastric aspirate was less than 5.0, an additional 30 milliliters of commercially available antacid (Maalox, Rorer) were administered. Cimetidine (400 mg per dose) and its placebo counterpart were administered every four hours for the first 10 days of the study. The route of administration was intravenous as long as an intravenous cannula was in place. Thereafter, the drugs were given orally, in doses equal to those given intravenously, provided the patient had adequate gastrointestinal motility.

The initial rate of administration of cimetidine and antacids was continued for the first 10 days. Thereafter, in the absence of sepsis, renal or respiratory failure, gastrointestinal hemorrhage or other significant stress, the administration of cimetidine was decreased to every six hours and antacid to one and three hours postprandially and at bedtime. All dosages were returned to the initial schedule with the recurrence of any of the aforementioned stress situations. Patients with renal insufficiency (serum creatinine > 3.5 mg/100 ml) were given cimetidine or its placebo at 12-hour intervals. The utilization of

antibiotics, hyperalimentation, ventilatory support, and other indicated treatment modalities was managed independently by the primary care physicians.

Endoscopy

Esophagogastroduodenoscopy was performed with an Olympus GIF-P2 fiberoptic panendoscope after topical anesthesia with 1% tetracaine. These examinations were performed on the day of admission and at three and 10 days after admission. Photographs and biopsies were taken to confirm endoscopic findings. Endoscopic findings in the esophagus, stomach, and duodenum were objectively coded according to the criteria listed in Table 1. This permitted a uniform categorization of the findings as to location, severity, and frequency. Endoscopic findings were further classified as being normal (no abnormalities seen), mild (erythema only), moderate (erosions), or severe (definite ulcerations).

Side Effects

Standard clinical evaluation and laboratory tests were obtained at predetermined intervals throughout the study. These tests included total white blood cell count, arterial blood gases, serum electrolytes, creatinine, phosphate, blood urea nitrogen, and liver functions. All stools and nasogastric aspirates were tested for occult blood. Daily stool counts, as well as clinical and x-ray signs of intestinal ileus, were also recorded. All patients reported were studied for a minimum period of 10 days.

Statistics

Differences in frequency of occurrence between treatment groups were tested using χ^2 with a single degree of freedom. Differences between means of continuous variables were tested by analysis of variance. In each instance, the null hypothesis was tested at a significance level of 5%. All data were recorded and objectively coded before terminating the study.

RESULTS

From July 1977 to March 1978, 40 patients were studied. Thirteen of these patients died from complications of their thermal injury prior to completion of the study. The remaining 27 patients (67.5%) completed at least 10 days of evaluation. Thirteen of these patients received cimetidine, and 14 were treated with antacids. The observed mortality for each treatment group was within the mortality limits expected for patients with the same extent of thermal injury. The data for age, burn size and mortality for both groups are shown in Table 2. There were no statistically significant differences in these comparisons.

Table 1. Endoscopy Data Code Sheet

1. Active Clinical Hemorrhage

- 0 - No
- 1 - Yes

2. Transfusions Required

- 0 - No
- 1 - Yes

3. Esophagus

- 0 - Negative examination
- 1 - Erythema, edema of mucosa only
- 2 - Erosions, single or multiple
- 3 - Ulceration(s)
- 4 - Active hemorrhage
- 5 - Probably secondary to nasogastric tube trauma

4. Stomach

- 0 - Negative examination
- 1 - Gastritis (mucosal congestion and erythema only)
- 2 - Gastritis with petechiae; no erosions
- 3 - Gastric ulcer, single
- 4 - Gastric ulcer, two or more
- 5 - Multiple erosions, superficial; no ulceration
- 6 - Findings in corpus only
- 7 - Findings in antrum or pylorus only
- 8 - Findings in both corpus and antrum
- 9 - Active hemorrhage present
- 10 - Probably secondary to nasogastric tube trauma

5. Duodenum

- 0 - Negative examination
 - 1 - Duodenal ulcer, single
 - 2 - Duodenitis (erythema and mucosal congestion)
 - 3 - Duodenal ulcer, two or more
 - 4 - Active hemorrhage
-

Table 2. Comparison of Therapeutic Groups

Treatment	No. of Pts	Age		Burn Size (% TBS*)		Mortality	
		Range	Average	Range	Average	No.	%
Cimetidine	13	19-72	38	41-79	54	6	46
Antacid	14	18-52	30	31-83	49	4	29

* TBS - total body surface.

Endoscopic Findings (Tables 3 and 4)

Eighty-one endoscopies were performed without complication on the 27 study patients. Mucosal biopsies confirmed endoscopic interpretations in six patients on cimetidine and four patients on antacids.

Esophageal disease was seen in five (38%) of the patients on cimetidine and seven (50%) of the patients on antacids. These abnormalities were mild (erythema only) in both groups and were almost always attributable to nasogastric tube trauma. Normal gastroduodenal findings as well as gastric erythema and gastric ulcer occurred with equal frequency in the two treatment groups throughout the course of observation. Twenty-three percent of the cimetidine group and 14% of the antacid group showed no abnormalities. In the cimetidine group, gastric erythema was seen in 77% and gastric ulcer in 23% of patients, with the same lesions in 71% and 14% respectively in the antacid group. Gastric erosion, however, was seen more frequently with antacid ($P < 0.05$).

Linear gastric erythema was the most frequent abnormal endoscopic finding and made up 16 (64%) and 17 (61%) of the total lesions in both groups, respectively. The next most frequent lesions in the cimetidine group were gastritis with petechiae, four (22%); gastric ulceration, three (12%); and multiple erosions, two (8%). The distribution for the antacid group was multiple erosions, eight (29%); gastric ulcer, two (7%); and gastritis with petechiae, one (4%).

In the cimetidine group, gastric abnormalities were located in the corpus 83% of the time and 78% of the time in the antacid group. The antrum was involved 50% and 43% of the time respectively for cimetidine and antacids. Duodenal disease was mild in every case and was present in only three patients (23%) on cimetidine and in four (29%) of the patients on antacids.

Natural History (Table 5)

Approximately 70% of all patients started the study with normal endoscopic findings. Each group had a comparable frequency of

Table 3. Frequency of Acute Gastric and Duodenal Disease in Relation to Size of Burns (Total Body Surface - TBS)

Extent of Injury (% TBS)	No. of Patients		No. with Gastric Abnormalities		No. with Duodenal Abnormalities	
	Cim.	Antacid	Cim.	Antacid	Cim.	Antacid
0-19	0	0	0	0	0	0
20-29	0	0	0	0	0	0
30-39	2	6	2	5	0	1
40-49	3	2	2	2	0	1
50-59	3	3	2	2	1	1
60-69	4	1	3	1	1	0
70-79	1	1	1	1	1	1
80-89	0	1	0	1	0	0
90-100	0	0	0	0	0	0
TOTALS	13	14	10 (76%)	12 (86%)	3 (23%)	4 (29%)

Table 4. Endoscopic Findings in 27 Patients with Thermal Injury

	No. of Patients (%)	
	Cimetidine	Antacid
Esophageal erythema	5 (38)	7 (50)
Gastric erythema	10 (77)	10 (71)
Gastric erosions	2 (15)*	8 (57)
Gastric ulcer	3 (23)	2 (14)
Duodenitis	3 (23)	4 (29)
Normal stomach and duodenum	3 (23)	2 (14)

* P < 0.05 versus Antacid group.

endoscopic abnormalities on day three. On day 10, four (30%) patients on cimetidine had abnormalities, while 11 (79%) patients on antacids had abnormalities ($P < 0.05$). Three patients on cimetidine (23%) and two patients on antacids (14%) had normal examinations throughout the 10-day period of observation.

Table 5. Natural History of Endoscopic Changes

	Day 0		Day 3		Day 10	
	Cim.	Antacid	Cim.	Antacid	Cim.	Antacid
Normal EGD*	9	10	3	4	9	3
Abnormal EGD	4	4	10	10	4	11
% Abnormal EGD	30	29	77	71	30 [†]	79

* EGD - Esophagogastroduodenoscopy.

† $P < 0.05$ versus Antacid group.

Side Effects (Table 6)

The groups did not differ significantly in mean serum creatinine, mean serum glutamic oxaloacetic transaminase or lowest total white blood cell count. Hypophosphatemia occurred with comparable frequency in both groups. The daily stool frequency was 0.8 on cimetidine and 1.7 on antacids ($P < 0.01$). Intestinal ileus, clinically evident and x-ray verified, occurred in three patients on antacids and one patient on cimetidine. One patient on cimetidine and three patients on antacids had at least one period of pure metabolic alkalosis as defined by arterial blood gas and serum electrolyte criteria. Only one case of metabolic alkalosis (see below) was felt to be clinically significant.

There were no major gastrointestinal complications (hemorrhage or perforation) in any patient during the study period. One patient on cimetidine had numerous gastric pH determinations of less than 5.0. Despite the absence of significant gastroduodenal lesions, he was removed from the study after a 10-day period of observation and treated with commercially available antacids. One patient on antacids developed metabolic alkalosis and colonic pseudo-obstruction while on the study. His study was discontinued after 18 days in order to define potential etiologies and facilitate management. Antacids were felt to be the most likely etiology of his alkalosis.

DISCUSSION

The major complications of stress ulceration occurring in thermally injured patients have been well controlled with antacid

Table 6. Side Effects

	Cimetidine	Antacid
Mean serum creatinine (mg/dl)*	1.5 ± 0.5	1.4 ± 0.4
Mean SGOT units*	48 ± 24	53 ± 26
Lowest WBC (X 10 ³)*	3.8 ± 1.6	3.7 ± 1.3
Stool frequency/day*	0.8 ± 0.5 [†]	1.7 ± 0.8
Hypophosphatemia (no. of days < 2 mg/dl)	2.8	5.6
Patients with ileus	1	3
Patients with metabolic alkalosis	1	3

* Mean ± Standard Deviation.

† P < 0.01 versus Antacid group.

therapy (2). Patients not treated with antacids develop gastroduodenal hemorrhage (> 3 units of blood over a 24-hour period) with a 22% frequency and gastric perforation with a 6% frequency (1). In 1975, the use of prophylactic antacids was started at the United States Army Institute of Surgical Research. Since then, there has been only one patient with major gastroduodenal hemorrhage in over 750 patients treated for thermal injury.

In the present study, both the frequency and distribution of gastric abnormalities were unchanged by either antacids or cimetidine from the frequency (78%) and distribution reported for untreated patients (1). There was, however, a two- to threefold reduction in gastric ulceration in both treatment groups when compared to Czaja's untreated controls (1). This reduction in severity of disease by both antacids and cimetidine, as well as the complete elimination of duodenal ulceration, was undoubtedly responsible for the cessation of major gastroduodenal complications. Since bleeding from erosive gastritis is rarely life-threatening in this patient population (1,10),

1. Czaja AJ, McAlhany JC, Pruitt BA Jr: Acute gastroduodenal disease after thermal injury: An endoscopic evaluation of incidence and natural history. *N Engl J Med* 291:925-929, 1974.

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the greater frequency of erosions seen in the antacid group did not alter that drug's ultimate efficacy.

Duodenal disease was mild (duodenitis only) and infrequent (approximately 25%) for both groups. This is a marked reduction when compared to that seen in the untreated group (72%) reported by Czaja (1). The duodenum seems very resistant to stress-induced disease when gastric acidity is therapeutically reduced. The persistent frequency and distribution of the gastric lesions imply only a relative reduction in mucosal susceptibility to injury.

Both antacids and cimetidine eliminated major gastroduodenal mucosal injury. In addition, the patients receiving cimetidine had a decreased incidence of gastric mucosal erosions compared to those patients receiving antacids. Since both antacids and H₂-receptor antagonists reduce gastric acid, further gastric mucosal protection afforded by cimetidine suggests other physiologic actions by that drug. Stress-induced gastric mucosal injury is potentiated by a combination of intraluminal acid, gastric mucosal barrier breakers, and alteration of gastric mucosal blood flow (11-13). Although histamine mediates changes in the transmucosal ionic flux produced by bile acid (14), cimetidine alone does not affect disruption of the mucosal barrier by sodium taurocholate (15). H₂-receptor antagonists have been shown to increase gastric mucosal blood flow (16). Furthermore, cimetidine has also been shown to confer a protective effect on gastric mucosal blood flow in hypotensive animals (17). These additional physiological effects of H₂-receptor antagonists may explain the

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reduction in gastroduodenal abnormalities seen on day 10 in the patients treated with cimetidine.

In this study, treatment with either cimetidine or antacids produced statistically identical changes in serum creatinine, serum glutamic oxaloacetic transaminase, total white blood cell count, and serum phosphate. Therefore, in the patient with severe thermal injury, cimetidine had no adverse effect on the liver, kidneys, or white blood cell count. The difference in stool frequency is statistically significant and may be important from the standpoint of overall patient management. Although there has been a report of increased ileus associated with H₂-receptor antagonists (18), our study does not support this association. Despite the lower stool frequency in the cimetidine group, there was no increased incidence of ileus in these patients. Thus, the favorable alteration in stool frequency by cimetidine does not appear to compromise intestinal motility. It is important to note that the side effects examined and discussed have all been associated with short term therapy. Chronic use of either treatment may potentiate additional side effects not discussed here (3,4,9).

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PRESENTATIONS AND/OR PUBLICATIONS

None

PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: GASTROINTESTINAL ALTERATIONS AND COMPLICATIONS IN
BURNED TROOPS -- ULCERATIVE GASTRIC DISEASE IN THE
SEPTIC BURNED RAT: A MODEL FOR THE STUDY OF NON-
HEALING STRESS ULCERS IN THE MILITARY POPULATION

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FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

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A spectrum of gastrointestinal lesions has been studied in the septic burned rat that appears to be morphologically similar to the erosive and ulcerative diseases that occur in human patients that have suffered various forms of stress, including sepsis, shock and trauma.

In burned rats infected topically with Pseudomonas aeruginosa, acute erosive and ulcerative gastric lesions, have been identified. The incidence, morphology and pathogenesis of these lesions have been investigated. Of the two ulcerative GI processes that occur in the rat, the first condition appears to be identical to the well-described, but poorly understood, stress ulceration. These lesions probably result from vascular or blood flow changes as well as from the effect of acid on compromised gastric mucosa. The second ulcerative process apparently occurs as a sequellae to hematogenous bacterial infection. These lesions are inflammatory in nature and always contain numerous gram-negative bacteria.

Burn Injury
Infection
Gastric Ulcers

GASTROINTESTINAL ALTERATIONS AND COMPLICATIONS IN BURNED
TROOPS -- ULCERATION GASTRIC DISEASE IN THE SEPTIC
BURNED RAT: A MODEL FOR THE STUDY OF NON-HEALING
STRESS ULCERS IN THE MILITARY POPULATION

Acute gastrointestinal ulceration has been associated with many types of stress and injury, including shock, sepsis, trauma, starvation, intracranial lesions, respiratory, renal and cardiac failure and burns (1). The number of predisposing factors suggests that the response of the gastric mucosa to injury is stereotyped and quite limited. An early form of the condition, superficial erosive disease of the stomach, was found in 83.5% of patients with burns larger than 30 TBS. These early superficial mucosal lesions are felt to progress into deep ulcerations which can cause serious sequelae of hemorrhage and occasionally perforation (2).

Despite the fact that practically every investigator in the field of gastrointestinal ulceration has noted the relationship between sepsis and stress ulceration, this association has not been adequately studied in an animal model. We have investigated gastrointestinal erosive and ulcerative disease in the septic burned rat.

METHODS

Male Charles River rats were subjected to a 25% total body surface scald burn and inoculated topically with Pseudomonas aeruginosa strain 59-12-4-4 (ISR). The inocula contained 1.7×10^7 bacteria. This model is uniformly fatal in the rat (3). Deaths are caused by invasive burn wound infection and sepsis. Hematogenous lesions are seen in many organs including the lung, kidney, adrenal, and spleen. Burned but uninfected rats served as controls. Initial experiments involved sequential sacrifice of rats during the course of infection. Other experiments were designed so that rats were examined immediately after they succumbed to the infection.

RESULTS

Experiment 1: A total of 44 burned Pseudomonas infected and control rats were examined to compare incidence of ulceration. Rats were examined at 2, 5, 7 and 9 days following burn and infection. Significant gastric mucosal lesions occurred only in the septic burned rats. None of the gastric lesions were felt to be hematogenous.

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Experiment 2: Twenty-five rats (500-600 g) were burned and challenged as in experiment #1. All rats died of Pseudomonas aeruginosa infection between days 8 through 21 post burn and were examined grossly and microscopically for gastroenteric lesions. Distribution of gastrointestinal lesions was as follows: Stress erosions or ulcers of the stomach - 9 rats and ulcerative lesions of small or large intestine - 7 rats. The gastric lesions were common in the rats that died between days 8 - 12. Microscopic evidence of ulcer healing was occasionally seen. The intestinal lesions occurred in rats in a random pattern between days 8 through 21.

Experiment 3: In a group of 30 rats (400-500 g) burned and infected with Pseudomonas aeruginosa, results were similar to experiment #2. Twelve rats had erosive or ulcerative gastric lesions and eleven had intestinal lesions. Distribution was somewhat different, however, with both the gastric and intestinal lesions occurring at a higher frequency in rats that died during the second week of the infection (Table 1).

Morphology of Gastrointestinal Lesions

The acute nonsuppurative mucosal lesions are most common in the glandular stomach. These necrotic and occasionally hemorrhagic mucosal defects are found by microscopy to extend in most cases only partially into the mucosa. They meet the morphologic criteria of "stress" erosions that have been well described in man and animals.

In contrast to the bland mucosal defects described above, the second type of lesion is usually a deep ulcerative mucosal defect which has a significant acute inflammatory infiltrate. Large numbers of gram-negative rod-shaped bacteria are seen deep in these ulcers and in several cases lesions of this type have caused functional perforation of the muscular intestinal wall with secondary bacterial serositis and peritonitis. As of now, our studies have not shown conclusively that all ulcerations of this type are truly hematogenous bacterial infections from the burn wound, but the finding of several lesions with similar morphology beneath intact gastric or intestinal mucosa supports this concept. Also, the microscopic appearance of the gastrointestinal lesions is strikingly similar to the hematogenous infections caused by Pseudomonas aeruginosa in other organs.

PUBLICATIONS/PRESENTATIONS: None.

TABLE 1: INCIDENCE AND LOCATION OF GASTROINTESTINAL LESIONS IN
PSEUDOMONAS-INFECTED BURNED RATS

<u>Day</u> <u>Post Burn</u>	<u>No.</u> <u>Examined</u>	<u>Site of Lesions</u>		
		<u>Stomach</u>	<u>Small Intestine</u>	<u>Large Intestine</u>
8	2	-	-	-
9	5	3	4	3
10	3	2	2	1
11	6	3	1	3
12	3	1	-	1
13	4	2	-	1
14	1	-	-	-
15	2	-	-	-
16	1	-	-	-
17	3	1	-	-

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Acidophilic intrasinusoidal liver bodies appearing in thermally injured patients at autopsy were examined by light microscopy utilizing several special stains and by scanning and transmission electron microscopy. Morphologically, these bodies were found to be membrane-bound protein material which were usually present in the hepatic sinuses but were also identified in hepatocytes, space of Disse and Kupffer cells. Correlation of clinical data with pathologic findings suggests that the intrasinusoidal bodies develop secondary to an allergic type response.

Liver
Bodies
Burn Patients
Electron Microscopy
Special Stains

GASTROINTESTINAL ALTERATIONS AND COMPLICATIONS IN BURNED TROOPS -- INTRASINUSOIDAL BODIES IN THE LIVER OF THERMALLY INJURED PATIENTS

Intracytoplasmic and intrasinusoidal bodies in the liver have been reported in a variety of disease processes in man (1,2,3,4,5) and animals (6,7,8,9,10). Recently, we have noted a morphologically unique intrasinusoidal body in postmortem liver specimens from some of our thermally injured patients. An extensive morphological evaluation of these bodies and comprehensive investigation of the clinical history in each case were performed to identify pathogenetic mechanisms surrounding the formation of the bodies.

METHODS

In all cases, liver tissue was obtained postmortem and fixed in 10% buffered formalin. Tissue for electron microscopy was washed in 0.1 M cacodylate buffer, post-fixed in 1% osmium tetroxide at 4° C and dehydrated in graded ethanols. Tissue for transmission electron microscopy was embedded in Epon 812. Thick sections (0.8 micron) for light microscopy were stained with Paragon multiple stain. Thin sections were doubly stained with uranyl acetate and lead citrate and examined in an RCA EMU 4 electron microscope. Tissue for scanning electron microscopy was further dehydrated in graded ethanol-Freon 113 solutions, dried by the critical point method in a Bomar SPC 900, coated with gold/palladium, and examined in an ETEC Autoscan electron microscope.

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8. Anderson PJ, Cohen S, Barka T: Hepatic injury. *Arch Pathol* 71:89-95, 1961.
9. Bassi M, Bernelli-Zazzera A, Cassi E: Electron microscopy of rat liver cells in hypoxia. *J Pathol Bact* 79:179-184, 1960.
10. Child PL, Ruiz A: Acidophilic bodies. *Arch Pathol* 85:45-50, 1968.

Tissue for light microscopy was processed according to conventional histologic techniques. All specimens were stained with hematoxylin and eosin (H&E). In addition, eight special stains were performed on tissue from each case.

The clinical course records of our subjects were examined for 32 predetermined indices that included both clinical and pathological findings, and all data were recorded for analysis.

RESULTS

After noting the liver intrasinusoidal bodies in two autopsies, a review of liver sections from all autopsies completed during a 5.5 year period (Table 1) was performed. A total of 312 autopsies was studied and six additional cases containing similar liver bodies were discovered. The total of eight positive samples was confined to the years 1975-1977. The incidence of occurrence of the bodies was 5.4% in 1975, 3.7% in 1976 and 9% for the first six months of 1977.

Table 1: Autopsies Reviewed and Positive Cases

Year	# Autopsies	# Positive Cases (%)
1967	47	0 (0%)
1968	36	0 (0%)
1974	79	0 (0%)
1975	74	4 (5.4%)
1976	54	2 (3.7%)
1977*	22	2 (9%)
TOTAL	312	8

*1 Jan to 30 Jun 77.

By light microscopy of H&E sections, the liver intrasinusoidal bodies appeared as mildly acidophilic, homogenous bodies about the size of, or larger than, free macrophages. In one of the patients, the average size was consistently smaller. The bodies were devoid of internal structure or nuclear material. In the Paragon stained plastic sections, the bodies had a similar morphology and were even more evident.

Selected special stains were done on all positive cases to determine the biochemical nature of these bodies. The eight stains used were periodic acid-Schiff (PAS), PAS with diastase, alcian blue, phosphotungstic acid hematoxylin (PTAH), oil Red-O, methyl green-pyronine (MGP), Giemsa, and Gomori iron. All eight special stains on all eight cases were negative thereby eliminating the presence of substances with available aldehyde groups, acidic sulfated muco substances, fibrin, collagen, neutral lipids, DNA, RNA, and iron.

The bodies were easily demonstrated by transmission electron microscopy and appeared as single membrane bound structures containing evenly distributed particulate, amorphous, moderately electron dense material. The bodies were free of any organized structures such as cellular organelles, inclusions, crystalline material, myelin figures, amyloid, or nuclear components. Membrane bound material identical to the intrasinusoidal bodies was occasionally seen in the cytoplasm of Kupffer cells and within the cytoplasm of hepatocytes. More frequently the same material could be found filling and expanding the space of Disse.

By scanning electron microscopy, the intrasinusoidal bodies appeared round to oval with a limiting membrane that was discontinuous creating a "moth-eaten" appearance. No cellular organelles could be visualized in the interior of the bodies. The bodies were numerous and often filled the liver sinuses.

The livers contained no consistent gross or histological changes (Table 2). The increased weights of the livers reflect the accelerated phase of liver growth documented experimentally by Arturson (11) and by Herndon and Panke (12).

Table 2: Liver Pathology of Positive Cases

Case #	Liver Weight	Microscopic
1	2,750 g	Mild congestion, Kupffer cell hyperplasia
2	2,275 g	Moderately acute passive congestion
3	??	Mild vascular congestion and fatty change
4	3,800 g	Moderate vascular congestion and dilation of sinusoids
5	218 g	Moderate vascular congestion
6	3,125 g	Mild general fatty change
7	2,360 g	Moderate acute congestion
8	3,250 g	Mild to moderate acute vascular congestion

?:Organs examined in-situ and biopsies taken due to limited autopsy permit.

Clinical records and postmortem protocols of the patients were thoroughly reviewed to determine a common factor which would explain the hepatic findings. Table 3 shows the indices examined. Liver function tests ranged from normal in some patients to a significant elevation in

11. Arturson G: Pathophysiological aspects of the burn syndrome with special reference to liver injury and alterations of capillary permeability. Acta Chir Scand Suppl 274:1-135, 1961.

12. Herndon D, Panke TW: Hepatomegaly in thermally-injured patients. Unpublished data.

TABLE 3. CLINICAL AND PATHOLOGICAL DATA

Case #	Age Sex Race	% TBSB/ J ²	Days PB	Anes.	Inhal. Inj.	Dopamine	HA	General Pathology
1	19MC	68.5/10	5	0	+	+ XI day	-	Burn wound sepsis, necrotizing broncho-pneumonia.
2	37C	72/62	22	1	-	+ XI days	-	Acute tubular necrosis, hematogenous pyelonephritis, severe acute pericarditis, aspiration pneumonia, mycotic burn wound invasion.
3	61MC	35/19	29	1	+	-	+	Diffuse severe myocardial fibrosis, aspiration pneumonia.
4	41MC	78/13	19	0	-	+ X2 days	-	Acute myocardial infarct, pituitary infarct.
5	64MFW	51/31	10	0	+	-	-	Burn wound sepsis, extensive hematogenous pneumonia.
6	24MC	47/0	6	0	-	+ X2 days	-	Burn wound sepsis.
7	21MC	54/35	8	1	+	+ XI day	-	Burn wound sepsis, moderate pulmonary hemorrhage.
8	26MC	68/30	31	0	+	+ X2 days	-	Suppurative thrombophlebitis, burn wound sepsis, severe hematogenous pneumonia.

Legend:
 + - Could not be determined
 % TBSB - % Total body surface burn
 J² - % Third degree
 Days PB - Days post burn survival
 Anes. - Anesthesia
 Inhal. Inj. - Inhalation injury
 HA - Hyperalimentation

selected serum values in other patients. Some serum values suggested hepatocellular necrosis while other values suggested intrahepatic cholestasis.

The microbiology data is summarized in Table 4. All but one patient

Table 4: Microbiology Results on Positive Cases

Case #	Blood Culture	# of Systemic Antibiotics	Topical Antibiotic	
			Sulfamylon	Silvadene
1	+	3	-	+
2	+	4	+	+
3	-	1	-	+
4	+	3	-	+
5	+	3	-	+
6	+	3	+	+
7	+	-	+	+
8	+	4	+	+

had one or more positive blood cultures involving one or more of six bacterial species (Pseudomonas, Staphylococcus, Streptococcus, Klebsiella, Enterobacter, Citrobacter) and one fungus (Candida). Likewise, all but one patient received systemic antibiotics involving one or more of the following: Keflin, Colymycin, Vancomycin, Gentamicin, Carbenicillin, Colistin, Ampicillin, Tobramycin, Cleocin, and penicillin. All eight of the patients received topical applications of silver sulfadiazine (Silvadene). Half of the patients were treated topically with Sulfamylon.

DISCUSSION

The importance of the liver in metabolism and detoxification strongly suggests that it plays a vital role in the individual's response to thermal injury. A variety of nonspecific morphological and physiological abnormalities have been demonstrated in the burn patient (11,12,13,14,15,16). Usually such changes are sublethal. The intrasinusoidal bodies in the liver reported in this paper, however, appear unique in the burn patient.

11. Arturson G: Pathophysiological aspects of the burn syndrome with special reference to liver injury and alterations of capillary permeability. *Acta Chir Scand Suppl* 274:1-135, 1961.
12. Herndon D, Panke TW: Hepatomegaly in thermally-injured patients. Unpublished data.
13. Czaja AJ, Rizzo TA, Smith WR Jr, Pruitt BA Jr: Acute liver disease after cutaneous thermal injury. *J Trauma* 15:887-894, 1975.
14. Chlumsky J, Dobias J, Vrabec R, Marecek B, Chlumska A, Matejicek V: Liver changes in burns, as seen in the clinical morphologic picture. *Acta Hepato-Gastroenterol* 23:118-124, 1976.
15. Teplitz C: Pathology of burns in Artz CP, Moncrief JA (Eds), *The treatment of burns*. London, Saunders, 1969.
16. Gilmore JP, Fozzard HA: Liver function following thermal injury. *Am J Physiol* 198:491-495, 1960.

The finding of two current cases with such sinusoidal bodies led to a retrospective search of autopsy material over a 5.5 year period which revealed similar bodies in six additional cases confined to a 2½ year period.

In spite of some autolytic changes in the tissue, transmission electron microscopy confirmed our impression that these bodies were not sloughed cells or cellular debris accumulated in the liver sinuses. They appeared, instead, to be membrane bound aggregates of proteinaceous material. The presence of the same material both in the Kupffer cells and in the cytoplasm of some of the hepatocytes suggests that this was a protein complex produced by the hepatocytes and extruded into the sinuses where it accumulated more rapidly than it could be removed by the Kupffer cells or normal blood flow. Scanning electron microscopy confirmed the large numbers and shape of the bodies in the liver sinusoids.

The negative results from the eight special stains performed effectively eliminated carbohydrate, lipids, DNA, RNA, iron, and fibrin as possible components of the intrasinusoidal bodies. These findings provided additional corroboration of the proteinaceous character of these bodies.

In selecting the 32 indices to consider in reviewing the clinical course records of the eight patients, we included the general physical characteristics of each patient, type and extent of burn, and numerous aspects of burn treatment as well as clinical laboratory, microbiology, and pathology data. After reviewing the recorded data, the only common factor was the use of topical silver sulfadiazine in all eight patients. This does not necessarily incriminate silver sulfadiazine as the causative agent producing the intrasinusoidal bodies as other factors may be acting in some combination to produce the bodies. Neither the extent of burn nor the postburn survival had any mediating effect on the quantity of the bodies present. It is noteworthy, however, that the bodies do not appear in any of the autopsy specimens reviewed until the introduction of silver sulfadiazine.

Several clinical studies have been done on the absorption and excretion of silver sulfadiazine in humans (17) and in pigs (18). Results have indicated that very small amounts of silver are absorbed through the burn wound, but considerably larger amounts of the sulfadiazine moiety are absorbed and excreted in the urine. No morphological study has been conducted in man to determine the effect silver sulfadiazine may have on tissue.

17. Fox CL: Silver sulphadiazine - a new topical therapy of pseudomonas infection. Arch Surg 96:184-188, 1968.

18. Lazare R, Watson PA, Winter GD: Distribution and excretion of silver sulphadiazine applied to scalds in the pig. Burns 1:57-64, 1974.

A number of histological and serological studies have been done on allergic liver reaction and injury in both man and experimental animals (19,20,21,22). Steiner (22) has demonstrated by electron microscopy the presence of antigen-antibody complexes in the liver that are morphologically similar to our intrasinusoidal bodies. This finding coupled with the known allergic response of some individuals to "sulfa" drugs suggests that the liver intrasinusoidal bodies may well be membrane bound protein complexes produced by the liver parenchyma as an allergic response.

19. McKinnon GE, Andrews EC Jr, Heptinstall RH, Germuth FG Jr: An immunohistologic study on the occurrence of intravascular antigen-antibody precipitation and its role in anaphylaxis in the rabbit. *Bull J Hopkins Hosp* 101:258-280, 1957.

20. Hartly G, Lushbaugh BS: Allergic focal necrosis of the liver. *Am J Pathol* 18:323-331, 1942.

21. Hawn CVZ, Janeway CA: Histological and serological sequences in experimental hypersensitivity. *J Exp Med* 85:571-590, 1947.

22. Steiner JW: Investigations of allergic liver injury. I. Light, fluorescent and electron microscopic study of the effects of soluble immune aggregates. *Am J Pathol* 38:411-436, 1961.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: GASTROINTESTINAL ALTERATIONS AND COMPLICATIONS IN BURNED
TROOPS--CIMETIDINE PREVENTS REDUCTION IN GASTRIC MUCOSAL
BLOOD FLOW DURING SHOCK

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
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1 October 1977 - 30 September 1978

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ABSTRACT

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This study evaluated cimetidine's possible role in regulating gastric mucosal blood flow in the anesthetized, stressed miniature swine. Stress consisted of hemorrhagic shock to a mean arterial pressure of 50mm Hg. Twenty-one animals were divided into three experimental groups: untreated controls, pre-shock cimetidine treatment, and post-shock cimetidine treatment. Gastric mucosal blood flows were determined (microsphere method) during a stabilization period and after 90 minutes of shock. Central hemodynamic indices were monitored throughout each experiment. In the fundus, mucosal blood flow decreased 59% in the controls, 11% in pre-shock, and 28% in post-shock cimetidine groups. Antral mucosal blood flow decreased 57% in controls, 19% in the pre-shock, and 33% in the post-shock cimetidine groups. In the corpal mucosa, blood flow decreased 53% in controls, 11% in the pre-shock group and 41% in the post-shock cimetidine group. Cimetidine administration, both pre and post-shock, conferred significant protection on mucosal blood flow changes related to shock. Pre-shock drug administration had a significantly greater protective effect than post-shock treatment on blood flow in the corpal mucosa.

Cimetidine
Piglets
Blood flow

CIMETIDINE PREVENTS REDUCTION IN GASTRIC MUCOSAL BLOOD FLOW DURING SHOCK

Several pathogenetic factors are believed to be interrelated to stress-induced gastric mucosal injury. Among these are gastric acidity, mucosal ischemia, bile reflux, and depressed mucosal energy. H_2 -receptor antagonists have been shown to decrease this injury^{1,2,3} and a correlation has been demonstrated between this protection and a reduction in gastric acidity.⁴ Whether these drugs act on other causative factors is not known. The purpose of this study was to investigate the effect of an H_2 -receptor antagonist, cimetidine, on gastric mucosal blood flow during hemorrhagic shock.

MATERIALS AND METHODS

Twenty-three miniature swine (Vita-Vet Laboratories, Marion, Ind.), weighing 9-12 kg each, had solid food withheld for 48 hours prior to experimentation. The animals were anesthetized (Chloralose 100mg/kg) and mechanically ventilated on room air with oxygen supplementation. Through a cervical incision, a 16 gauge polyethylene catheter was advanced retrograde down the right common carotid artery into the left ventricle, and secured in place for microsphere injection. Bilateral inguinal incisions were made and both femoral arteries and the left femoral vein were cannulated with 16 gauge catheters. Both arterial catheters were advanced into the distal aorta. The left sided arterial catheter was connected to a pressurized, heparinized reservoir which allowed controlled hemorrhage with maintenance of a specific arterial blood pressure. The right femoral catheter was connected to a Statham P23Db transducer (Statham Instruments, Oxnard, Ca.) to record systemic arterial blood pressure. The left femoral vein catheter was used for drug infusion. A three channel, 5Fr Swan-Ganz thermodilution catheter was advanced via the right femoral vein into the pulmonary artery. The central venous pressure and pulmonary arterial pressure channels were connected to Statham P23Db transducers. The Swan-Ganz thermister circuitry was coupled to a computer for cardiac output determinations (Instrument Laboratories, Chicago, Ill.) at 30 minute intervals. All transducers were coupled to a Beckman RM dynograph recorder (Beckman Instruments, Schiller, Park, Ill.) for continuous monitoring. Arterial

1. Bugajski J, Hano J and Danek L: Effect of metiamide, a histamine H_2 -receptor antagonist, on the development of gastric stress ulcers and acid secretion. *Eur J Pharmacol* 36:237, 1976.
2. Levine BA, Teegarden DK, McLeod CG et al: Cimetidine prevents stress-induced gastric erosions. *Surg Forum* 28:359, 1977.
3. Shirazi S, Foster LD and Hardy BM: The effect of metiamide, an H_2 -receptor antagonist in the prevention of experimental stress ulcers. *Gastroenterology* 71:421, 1976.
4. Levine BA, Sirinek KR, Teegarden DK et al: Effect of cimetidine on gastric secretory function during stress. *J Surg Res* (in press).

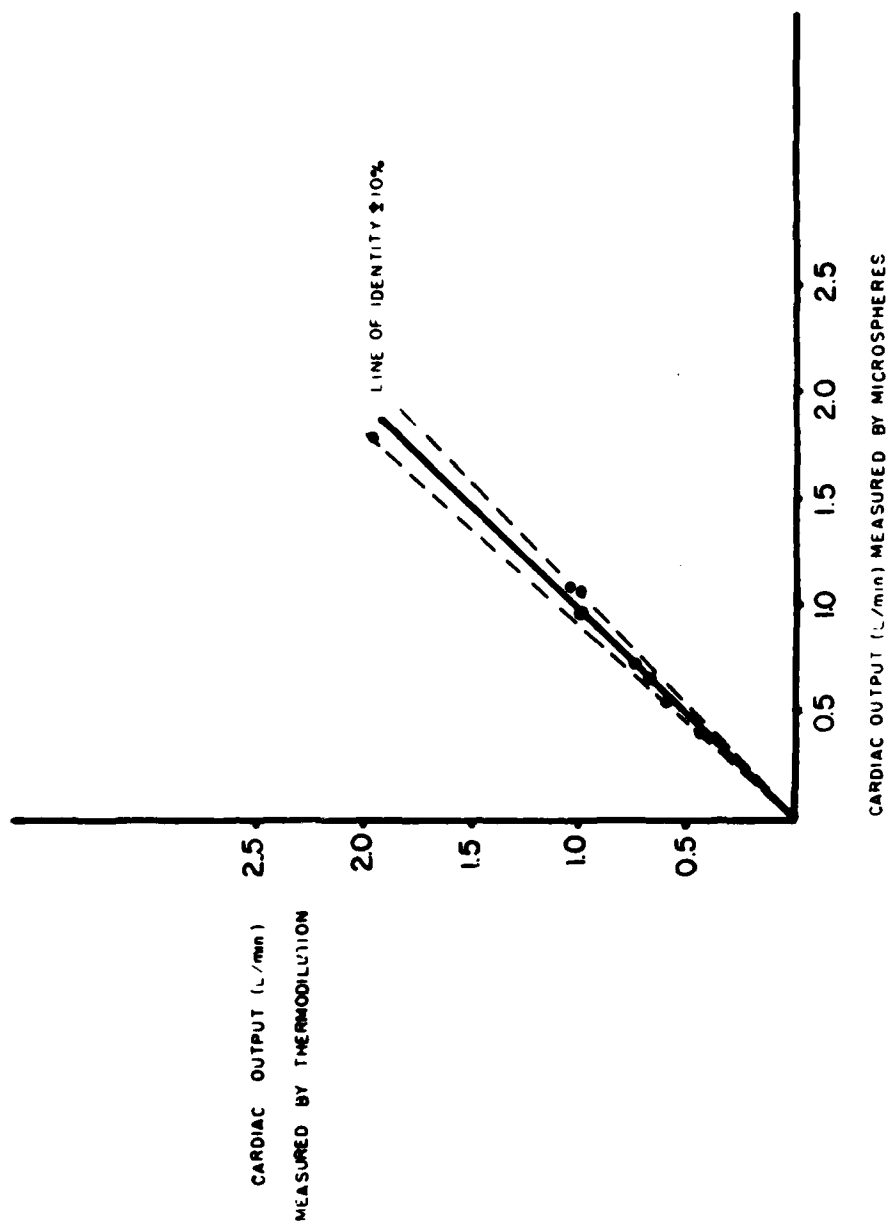


FIG. 1 Comparison of cardiac outputs determined simultaneously by thermodilution and microsphere techniques.

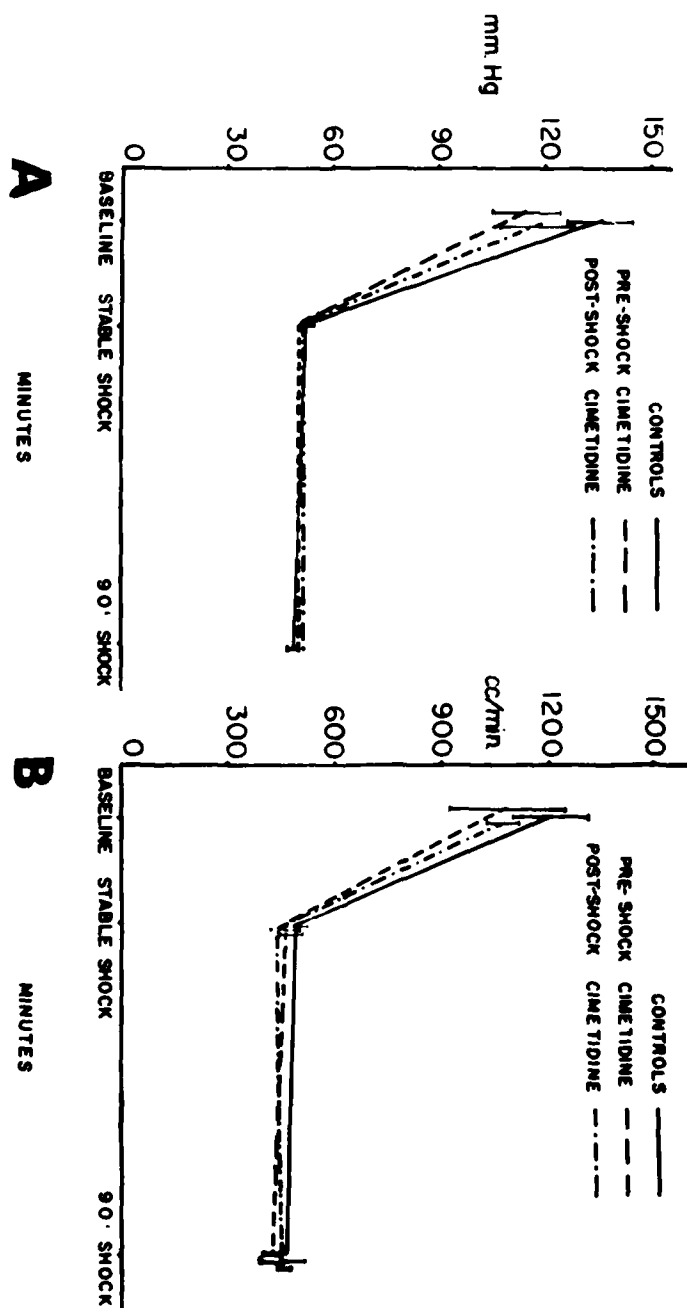


FIG. 2 Mean systemic arterial pressures (A) and cardiac outputs (B) of the three experimental groups.

blood samples were drawn every 45 minutes for determination of PaO_2 , PaCO_2 , and PH. PaO_2 was maintained at a level of 100mm Hg and PaCO_2 at 30-40mm Hg by adjusting oxygen inflow and/or respiratory rate.

Microspheres (3M Company, St. Paul, Minn.) 15 ± 5 μ in diameter, labelled with ^{125}I , ^{141}Ce , ^{85}Sr , or ^{46}Sc were suspended in 10% dextran and Tween (1 drop/10ml). For each determination, approximately 1.2×10^6 microspheres were mechanically agitated and injected as a bolus into the left ventricle. The cannula was then flushed immediately with five milliliters of 5% dextrose in water at body temperature. Cardiac output was determined immediately before and after injection.

Upon completion of each experiment, the animal was sacrificed by injection of potassium chloride. The stomach was removed and divided into fundic, corporal and antral portions. The mucosa and submucosa were dissected from the muscular and serosal layers, cut into pieces of approximately equal size, placed in pre-weighed glass tubes, and weighed to the nearest milligram. The specimens were then counted for two minutes in a Packard Auto Gamma Scintillation Spectrometer (Packard Instruments, Downer's Grove, Ill.). Energy window settings on the counter were: ^{125}I , 30 to 115 keV; ^{141}Ce , 115 to 200 keV; ^{85}Sr , 420 to 590 keV; and ^{46}Sc , 790 to 1160 keV. Separation of the individual isotopic counts, with accommodation for interference by the other radioactive labels, was accomplished by the use of standard techniques.⁵

Mucosal blood flows were calculated by the formula:

$$\text{Flow (ml/min/100g tissue)} = \frac{(\text{Cardiac output}) (\text{Counts/min/100g tissue})}{\text{Total counts injected}}$$

Microsphere mixing in the left ventricle was tested in two animals by simultaneous determinations of cardiac outputs using both thermal-dilutional and microsphere reference sample techniques. Starting 20 seconds prior to microsphere injection and continuing until 100 seconds after injection, reference blood was withdrawn from the distal aorta at a rate of 3.0 milliliters per minute, placed in pre-weighed tubes, weighed (1 ml. blood = 1.05g), and counted. Microsphere cardiac output was calculated by the formula:

$$\text{Cardiac Output (ml/min)} = \frac{(\text{Reference Flow}) (\text{Total Counts Injected})}{\text{Reference Sample Counts}}$$

A total of eight paired determinations were made, ranging from 350 ml/min to 1900 ml/min (Fig 1). All points fell within $\pm 10\%$ of the line of identity.

The remaining 21 animals were divided into three equal groups. All animals underwent a stabilization period of at least 45 minutes after placement of catheters. Each animal underwent an injection of microspheres at the end of that period.

5. Heyman MA, Payne BD, Hoffman JI et al: Blood flow measurements with radionuclide-labeled particles. Prog Cardiovasc Dis 20:55, 1977.

Seven control animals were then slowly bled until a stable mean systemic arterial pressure of 50 mm Hg was attained. Shock was maintained for 90 minutes, after which another microsphere injection was made and the animal sacrificed. The second group of animals received cimetidine, 10 mg/kg, IV bolus, after stabilization and microsphere injection was made. The animals were then hemorrhaged and, after 90 minutes of shock, a third microsphere injection was made and the animals sacrificed. The third group underwent hemorrhagic shock following stabilization and microsphere injection. When the mean arterial pressure was steady at 50 mm Hg, cimetidine (10 mg/kg, IV bolus) was given. Shock was continued for 90 minutes, after which another microsphere bolus was injected and the animal sacrificed.

Analysis of variance was used to compare changes in mean arterial pressures and cardiac outputs between experimental groups. Student's paired t-test was employed to compare mucosal blood flows within the control and post-shock cimetidine groups. Scheffe's⁶ modification of analysis of variance was used to compare the three mucosal blood flow determinations in the pre-shock cimetidine group. Changes in mucosal blood flow, from stabilization to 90 minutes of shock, were compared among the three experimental groups by the Kruskal-Wallis⁷ one way analysis of variance.

RESULTS

Central Hemodynamics: A significant decrease in mean systemic arterial pressure (Fig. 2) from the stabilization period through 90 minutes of shock occurred in all treatment groups: control group ($\downarrow 64\%$), pre-shock cimetidine group ($\downarrow 57\%$), and post-shock cimetidine group ($\downarrow 57\%$). A significant decrease in cardiac output (Fig. 2) between stabilization and shock also occurred in all treatment groups: controls ($\downarrow 62\%$), pre-shock cimetidine group ($\downarrow 63\%$), and post-shock cimetidine group ($\downarrow 59\%$). Arterial pressure and cardiac output changes among the three groups were not significantly different.

Fundic Mucosal Blood Flows: Fundic mucosal blood flows in the three experimental groups are presented in Fig. 3. In the untreated controls, baseline (stabilization period) mucosal blood flow was 14.0 ± 1.6 ml/min (mean \pm SEM) and dropped by 59% to 5.8 ± 1.6 ml/min, at 90 minutes of shock ($p < 0.05$). Baseline mucosal blood flow in the pre-shock cimetidine group was 15.6 ± 2.7 ml/min and was not altered significantly by the addition of cimetidine prior to hemorrhage. After 90 minutes of shock the mucosal blood flow, 13.9 ± 2.0 ml/min, had decreased by 11% but remained statistically unchanged from baseline values. The baseline blood

6. Ostle B: Statistics in Research, Ames, Iowa, 1963, Iowa State University Press.

7. Siegel S: Non Parametric Statistics for the Behavioral Sciences, New York 1956, McGraw-Hill.

flow in the post-shock cimetidine group was 18.0 ± 3.7 ml/min and decreased by 28% to 12.9 ± 2.7 ml/min ($p < 0.05$). When the per cent decrease in fundic mucosal blood flow for each experimental group was compared to the others, there was a significant difference between the controls and both cimetidine treated groups ($p < 0.02$). No difference between the pre and post-shock cimetidine groups could be discerned ($p = \text{NS}$).

Corpal Mucosal Blood Flows: Mucosal blood flows in the gastric corpus are depicted in Fig. 4. In the control group, flow decreased 53% from a baseline of 16.3 ± 1.2 ml/min to 7.6 ± 1.9 ml/min ($p < 0.01$). Baseline flow in the pre-shock cimetidine group was 16.4 ± 1.7 ml/min. Addition of cimetidine prior to hemorrhage resulted in a flow of 18.9 ± 3.0 ml/min ($p = \text{NS}$ vs baseline). After 90 minutes of shock, the mucosal blood flow had decreased by 11% from the baseline value to 14.5 ± 1.6 ml/min ($p = \text{NS}$). In the post-shock cimetidine group, baseline mucosal blood flow was 20.6 ± 3.7 ml/min. The flow rate at 90 minutes of shock decreased by 41% 20.6 to 12.1 ± 1.1 ml/min ($p < 0.05$). The per cent decrease in the corpal mucosal blood flow in both cimetidine treated groups differed significantly from that of the controls ($p < 0.05$). The pre-shock cimetidine group had a significantly smaller reduction in mucosal blood flow than did the post-shock cimetidine group ($p < 0.05$).

Antral Mucosal Blood Flows: Fig. 5 presents the antral mucosal blood flows for each of the three experimental groups. In the control group a 57% drop in flow occurred, from a baseline value of 16.0 ± 0.8 ml/min to a 90 minute shock value of 6.8 ± 2.7 ml/min which was not significantly changed following the administration of cimetidine. At 90 minutes of shock, the flow rate had diminished to 13.6 ± 1.3 ml/min. Although this was a 19% decrease from the baseline value, the change was not significant. The post-shock cimetidine group of animals sustained a decrease of 33% in its mucosal flow, from a baseline value of 17.5 ± 2.4 ml/min to a 90 minute shock value of 11.7 ± 1.2 ml/min ($p < 0.05$). Comparison of the mucosal blood flow changes among each of the experimental groups showed both cimetidine treated groups to differ significantly from the controls ($p < 0.02$). There was no difference in mucosal blood flow change between the pre and post-shock cimetidine groups ($p = \text{NS}$).

DISCUSSION

Information on H_2 -receptor antagonists appeared first in 1972 in a report by Black.⁸ These drugs competitively inhibit the action of histamine at the H_2 -receptor in the gastric mucosa, thereby decreasing gastric acid output. Their ability to control both resting and stimulated gastric acidity has led to their use in hyper-secretory states such as peptic ulcer disease and the Zollinger-Ellison syndrome.

H_2 -receptor antagonists have also been used to decrease gastric

8. Black JW, Duncan CJ, Durant CR et al: Definition and antagonism of histamine H_2 -receptors. Nature 236:385, 1972.

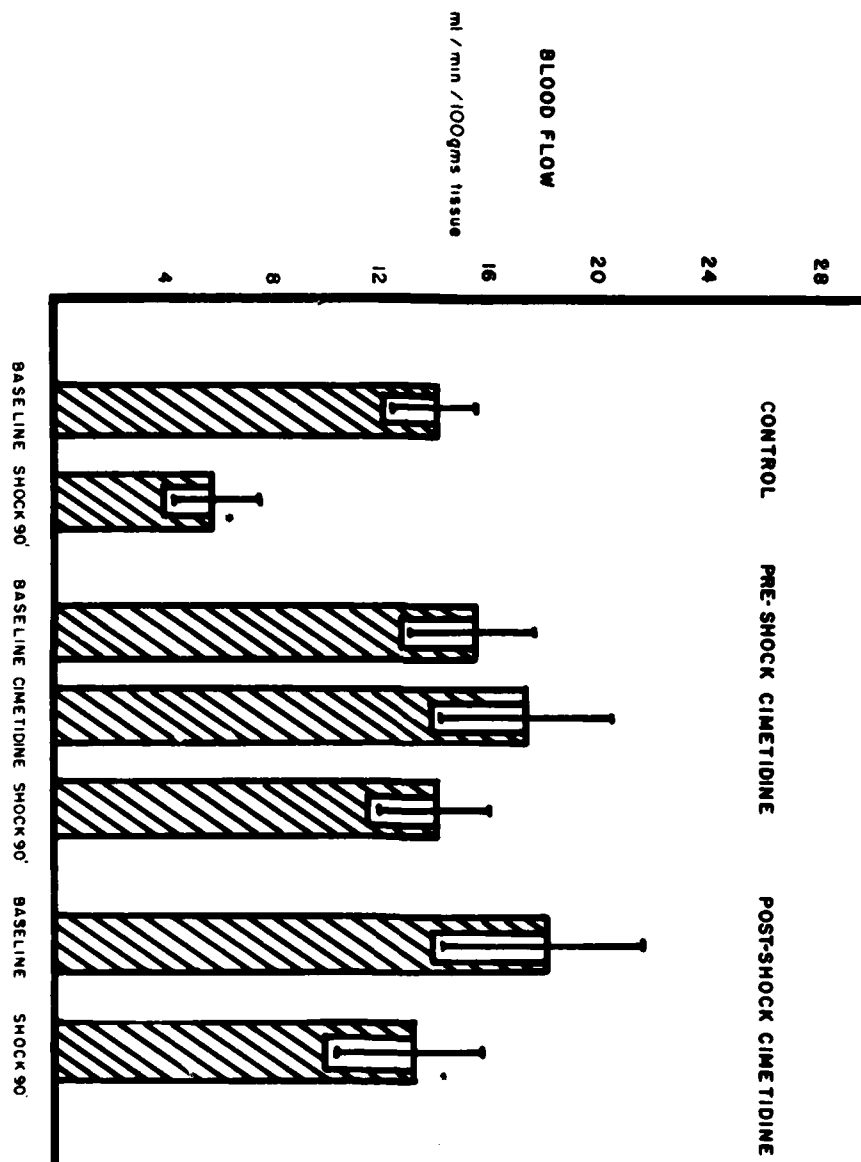


FIG. 3 Fundic mucosal blood flows of the three experimental groups (mean \pm SEM). (*Denotes statistical significance vs. baseline blood flow.)

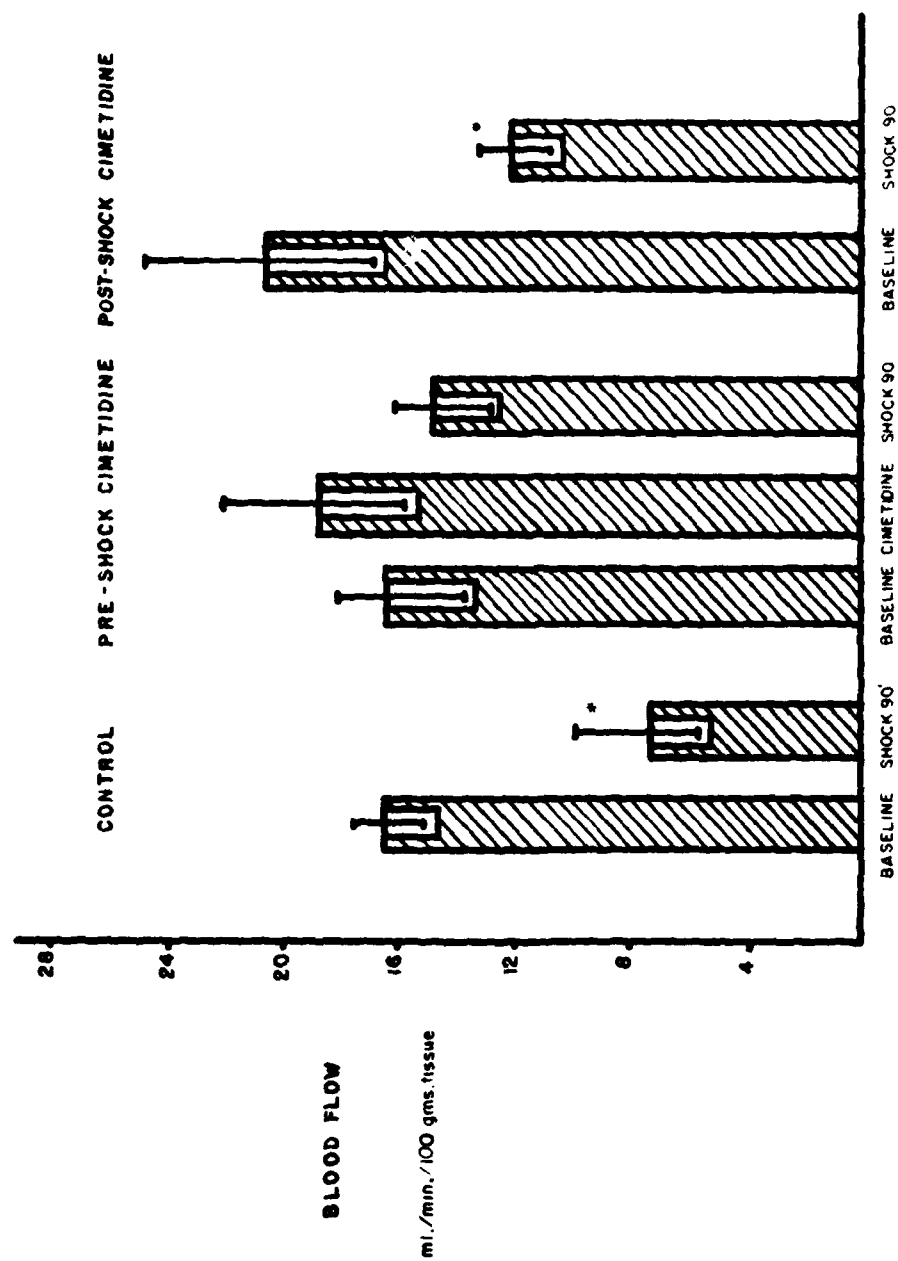


FIG. 4 Corpal mucosal blood flows of the three experimental groups (mean + SEM). (*Denotes statistical significance vs. baseline blood flow.)

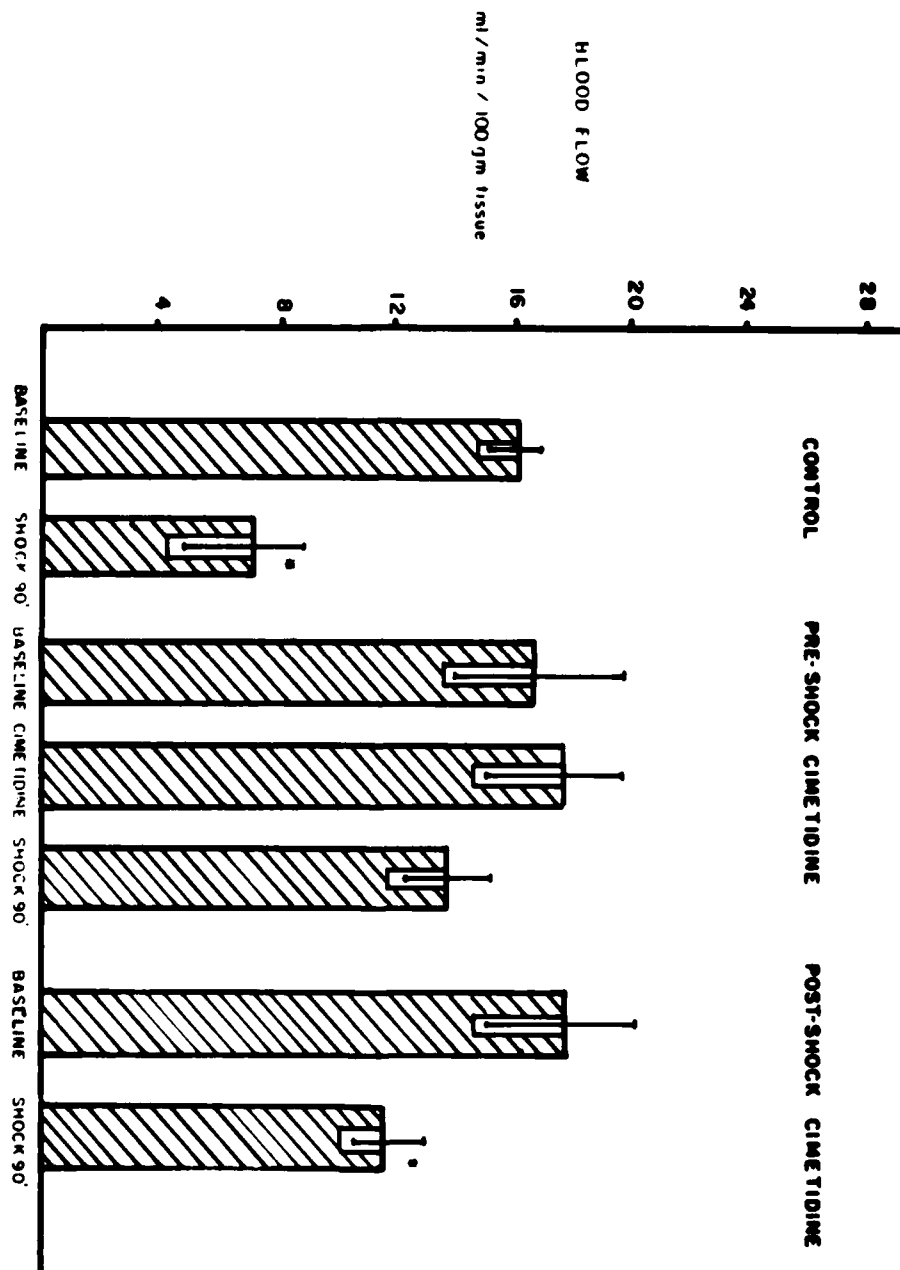


FIG. 5 Antral mucosal blood flows of the three experimental groups (mean \pm SEM). (*Denotes statistical significance vs. baseline blood flow.)

mucosal injury related to stress. Metiamide was utilized successfully by Bugajski¹ in a stressed rat model. Shirazi³ investigated a canine hemorrhagic shock model in which taurocholate and acid were added to the stomach and found the animals treated with metiamide to have significantly fewer gastric erosions than controls.

A cold restraint rat model has been used in our laboratory² to demonstrate the effectiveness of cimetidine in decreasing gastric mucosal injury caused by stress. Cimetidine conferred a similar degree of protection on animals undergoing stress which had been augmented by aspirin or taurocholate ingestion. Further experiments in the same model⁴ showed that this drug's protective effect was related to its ability to decrease the total output of gastric acid below an already depressed level in stress. However, this does not mean that a direct cause and effect relationship exists. It is possible that H₂-receptor antagonists protect the stressed gastric mucosa in other ways and that their ability to lower gastric acid output is only part of their physiologic action.

Moody⁹ has emphasized the interaction of multiple factors in the genesis of stress-related gastric injury. Principal among these causes is a decrease in mucosal blood flow. Mersereau¹⁰, using a hemorrhagic shock rat model, observed gastric mucosal ischemia in areas which subsequently ulcerated. Other investigators^{11,12} have described a correlation between decreased gastric mucosal blood flow and erosive damage in other animal models. Thus, with blood flow occupying such a central position in stress-ulcer pathogenesis, and H₂-receptor antagonists conferring protection against those ulcers, it was intriguing to investigate the drug's possible actions on blood flow.

Investigators^{13,14} have demonstrated the ability of H₂-receptor antagonists to decrease gastric mucosal blood flow in intact² animals whose

9. Moody FG, Cheung LY, Simons MA et al: Stress and the acute gastric mucosal lesion. *Am J Dig Dis* 21:148, 1976.

10. Mersereau WA and Hinchey EJ: Effect of gastric acidity on gastric ulceration induced by hemorrhage in the rat, utilizing a gastric changer technique. *Gastroenterology* 64:1130, 1973.

11. Richardson RS, Norton LW, Sales JE et al: Gastric blood flow in endotoxin-induced stress ulcer. *Arch Surg* 106:191, 1973.

12. Shirazi S, Mueller TM and Hardy BM: Canine gastric acid secretion and blood flow measurement in hemorrhagic shock. *Gastroenterology* 73:75, 1977.

13. Cheung LY and Lowry SF: Canine gastric blood flow and oxygen consumption during cimetidine inhibition of acid secretion. *Surg. Forum* 27:390, 1976.

14. Konturek SJ, Tasler J, Obtulowicz W et al: Effect of metiamide, a histamine H₂-receptor antagonist, on mucosal blood flow and serum gastrin level. *Gastroenterology* 66:982, 1971.

blood flow was first increased with histamine, pentagastrin, or urecholine. Olsen¹⁵ found that cimetidine given to a rat in the unstimulated state did not alter mucosal blood flow significantly from control values. Main,¹⁶ using a similar model, found that burimamide increased gastric mucosal blood flow 98% over control values. However, none of the investigations cited have studied H_2 -receptor antagonism in shock, when gastric mucosal blood flows are known to be decreased.

Radioactively labelled microspheres were used in our experiments to measure regional blood flow. Spheres, 15u in diameter, have been shown¹⁷ to lodge in the gastric microvasculature without passing into the venous circulation. Those studies, in the chambered, isolated dog stomach, demonstrated a strong correlation between flows determined by microspheres and venous effluent collection. Accurate flows were also measured by several different microspheres whether they were injected simultaneously or serially, making this technique suitable for repeated flow determinations during an experiment.

While some investigators use arterial reference samples to calculate flow,^{11,12} others depend on independent measurements of cardiac output multiplied by a ratio of regional radioactivity to total radioactivity injected.⁵ The latter method is based on an assumption of uniform mixing of spheres in the left ventricle assuring their delivery to a specific organ in proportion to cardiac output. This assumption must be proven for each experimental model by comparing microsphere derived cardiac outputs with those independently measured. Heyman⁵ has reported excellent correlation between microsphere cardiac outputs and those derived by electromagnetic flowmeter, Fick, and green dye methods. For the miniature swine shock model used in the present studies, we have found good correlation between microsphere and thermodilution methods of cardiac output measurement (Fig 1).

In our study, hemorrhagic shock resulted in a diminished overall gastric mucosal blood flow in untreated, control animals. This 57% decrease from the stabilization level paralleled a 62% drop in cardiac output and demonstrated that the gastric mucosa was not spared during shock. In the unshocked, normotensive pig cimetidine did not significantly alter gastric mucosal flow (Fig 3,4,5). This finding agrees with Olsen's¹⁵ observations in the rat model.

Both cimetidine treatment regimens offered a significant degree of protection from the shock-related fall in gastric mucosal blood flow

15. Olsen CO and Moody FG: Mechanism of histamine-resistant gastric acid secretion in rats. *Surg Forum* 27:388, 1976.

16. Main IHM and Whittle BJR: A study of the vascular and acid-secretory responses of the rat gastric mucosa to histamine. *J Physiol* 257:407, 1976.

17. Archibald LH, Moody FG and Simmons M: Measurement of gastric blood flow with radioactive microspheres. *J Appl Physiol* 38:1051, 1976.

observed in the untreated pigs. In animals receiving cimetidine post-shock, overall gastric mucosal blood flow decreased 34% from the stabilization level while cardiac output dropped 59% during the same time period. Similarly, in the animals receiving cimetidine prior to hemorrhage, overall mucosal blood flow in shock decreased only 14% from stabilization values. During the same period, cardiac output was diminished by 63%.

Both pre and post-shock cimetidine treatment offered the same degree of protection from shock-related blood flow changes in the antral and fundic segments of the gastric mucosa. It is of interest that pre-shock cimetidine conferred a significantly greater degree of protection on the corpal mucosa than did post-shock drug administration. In this regard, other authors^{11,12} have noted a greater sensitivity of the corpal mucosa to shock and subsequent ulceration.

Finally, the results of this study demonstrate that cimetidine's role in decreasing stress-induced gastric injury may not be due solely to its ability to reduce gastric acid secretion. Even decreased amounts of gastric acid will not be well tolerated by an ischemic mucosa. By sparing gastric mucosal blood flow during shock, cimetidine may allow the gastric mucosa to resist the acid present in the stomach.

PRESENTATIONS

Levine BA: Cimetidine prevents reduction in gastric mucosal blood flow during shock. Presented at the Thirty-ninth Annual Meeting of the Society of University Surgeons, Louisville, KY, Feb. 9-11, 1978.

PUBLICATIONS

Levine BA, Schwesinger WH, Sirinek KR, Jones D, and Pruitt BA Jr: Cimetidine prevents reduction in gastric mucosal blood flow during shock. *Surgery* 84:113-119, July 1978.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				DA OG 6974		78 10 01		78 10 01	
1. DATE PREVIOUSLY	2. DATE OF CHANGE	3. SECURITY CLASS	4. SECURITY CLASS	5. SECURITY CLASS	6. SECURITY CLASS	7. SECURITY CLASS	8. SECURITY CLASS	9. SECURITY CLASS	10. SECURITY CLASS
77 10 01	D. CHANGE	U	U	U	U	U	U	U	U
11. NO. CODES		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER		NCOMP UNIT NUMBER	
A. PRIMARY		61102A		35161102BS05		00		084	
B. CONTRIBUTING									
C. CONTRIBUTING									
12. TITLE (Provide with Security Classification Code) (U) Alterations in Pulmonary Function and Pulmonary Complications in Burned Soldiers (44)									
13. SCIENTIFIC AND TECHNOLOGICAL AREA 003500 Clinical Medicine									
14. START DATE 76 10			15. ESTIMATED COMPLETION DATE Cont			16. FUNDING AGENCY DA		17. PERFORMANCE METHOD C. In-House	
18. CONTRACT GRANT Not Applicable				19. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS		B. FUNDS (in thousands)	
A. DATES/EFFECTIVE				EXPIRATION		FISCAL YEAR		CURRENT	
B. NUMBER				C. TYPE		78		1.7	
C. TYPE				D. AMOUNT		79		1.3	
D. KIND OF AWARD				E. CUM. AMT.		56		50	
19. RESPONSIBLE OGD ORGANIZATION				20. PERFORMING ORGANIZATION					
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research					
ADDRESS: Ft Sam Houston, Texas 78234				ADDRESS: Pulmonary Section Ft Sam Houston, Texas 78234					
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)					
NAME: Basil A. Pruitt, Jr., COL, MC				NAME: Victor Lam, MAJ, MC					
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-6532					
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:					
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS					
				NAME:					
				NAME:					
22. KEYWORDS (Provide EACH with Security Classification Code) (U) Burn injury; (U) Pulmonary function tests; (U) Humans; (U) Goats; (U) Total thoracic resistance; (U) Arterial blood gas tension									
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Provide text of each with Security Classification Code.)									
<p>23. (U) To document the site of inhalation injury early in the clinical course and to follow its progression quantitatively. To develop a goat model of inhalation injury with plastic polymers (toluene di-isocyanate) as the agent of injury.</p> <p>24. (U) Total respiratory system will be measured by the oscillometrics method. Polyurethane foam will be ignited to produce inhalation injury in tracheostomized goats. Serial measurements of pulmonary function and pathologic studies will be obtained.</p> <p>25. (U) 7710 - 7809 On-line computer computation of total thoracic pulmonary resistance is operational, with a coefficient of variation of 10%. Fourteen subjects with extrinsic asthma have been tested to date during routine intradermal skin tests. The total respiratory resistance follows that of a bronchial-provocation challenge with antigen. It is concluded that total thoracic resistance measurement during skin testing can replace inhalation bronchial challenges for extrinsic asthmatics. Measurements on thermally injured soldiers await computer capability on the hospital ward.</p>									

*Available to contractors upon originator's approval

DD FORM 1498

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TERMINATION

PROJECT NO. 35161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: ALTERATIONS IN PULMONARY FUNCTION AND PULMONARY COM-
PLICATIONS IN BURNED SOLDIERS -- EVALUATION OF UPPER
AIRWAY OBSTRUCTION IN THERMAL INJURY BY FORCED
OSCILLATIONS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

Victor Lam, M.D., Major, MC
Cleon W. Goodwin, Jr., M.D., Major, MC
Edwin W. Hander, M.A.
Douglas E. Mills, First Lieutenant, MSC
SP5 Dianne L. Martin

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: ALTERATIONS IN PULMONARY FUNCTION AND PULMONARY COMPLICATIONS IN BURNED SOLDIERS -- EVALUATION OF UPPER AIRWAY OBSTRUCTION IN THERMAL INJURY BY FORCED OSCILLATIONS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

Investigators: Victor Lam, M.D., Major, MC
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Reports Control Symbol MEDDH-289(R1)

Upper airway obstruction following inhalation injury can be a clinically silent event. Currently, direct visualization of the larynx is the only accurate method to detect upper airway obstruction. However, it is an invasive procedure and does not allow serial observations readily or quantitative assessment of airway closure.

Total respiratory resistance can determine the degree of laryngeal closure. On-line computer computation of resistance is operational with a coefficient of variance of 10%. A fast Fourier transform is performed on mouth pressure and mouth flow signals to determine total respiratory resistance and phase angle relationship.

In order to evaluate the operational capabilities of the on-line measurement of pulmonary resistance, 17 extrinsic asthmatics were studied during intradermal skin testing.

	Baseline	30 min	60 min	90 min
5 Hertz	1.00 \pm 0.01	1.18 \pm 0.06	1.11 \pm 0.05	1.11 \pm 0.05
11 Hertz	1.00 \pm 0.01	1.24 \pm 0.05	1.22 \pm 0.06	1.11 \pm 0.04

Normalized to baseline \pm SEM

Results parallel those of an inhalation challenge test for bronchial provocation. No significant changes occurred for subjects with allergic rhinitis or intrinsic asthma.

Collection of data on total respiratory resistance in inhalation injury awaits the availability of computer facilities on the hospital ward.

Burn injury
Pulmonary function tests
Total thoracic resistance
Upper airway obstruction

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION# DA OG 6971		2. DATE OF SUMMARY 78 10 01		3. REPORT NUMBER DD FORM 1498-1, 1 MAR 68	
4. DATE PREVIOUS SUMMARY 77 10 01	5. KIND OF SUMMARY D. CHANGE	6. SUMMARY ACTIVITY U	7. WORK SECURITY U	8. REGRADING NA	9. USER INSTN NL	10. SPECIFIC DATA CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO		11. LEVEL OF SUM A. WORK UNIT	
12. NO. CODES		13. PROGRAM ELEMENT		14. PROJECT NUMBER		15. TASK AREA NUMBER		16. WORK UNIT NUMBER	
a. PRIMARY		61102A		3S161102BS05		00		091	
b. 62774A		62774A		3S162774A820		00		115	
c. CONTRIBUTING									
17. TITLE (Precede with Security Classification Code) (U) Evaluation of Burn Wound Care in Troops With Burn Injury (44)									
18. SCIENTIFIC AND TECHNOLOGICAL AREAS 003500 Clinical Medicine									
19. START DATE 76 10		20. ESTIMATED COMPLETION DATE Cont		21. FUNDING AGENCY DA		22. PERFORMANCE METHOD C. In-House			
23. CONTRACT GRANT Not Applicable				24. RESOURCES ESTIMATE PRECEDING FISCAL YEAR 78		25. PROFESSIONAL MAN YRS 2.6		26. FUNDS (In thousands) 57	
27. CATES/EFFECTIVE D. NUMBER C. TYPE		28. EXPIRATION 4. AMOUNT F. CUM. AMT.		29. CURRENT 79		4.5		98.5	
30. RESPONSIBLE DCD ORGANIZATION NAME: US Army Institute of Surgical Research ADDRESS: Ft Sam Houston, Texas 78234 RESPONSIBLE INDIVIDUAL NAME: Basil A. Pruitt, Jr, COL, MC TELEPHONE: 512-221-2720				31. PERFORMING ORGANIZATION NAME: US Army Institute of Surgical Research Clinical Division ADDRESS: Ft Sam Houston, Texas 78234 PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution) NAME: Richard C. Treat, MAJ, MC TELEPHONE: 512-221-6532 SOCIAL SECURITY ACCOUNT NUMBER: ASSOCIATE INVESTIGATORS NAME: NAME: DA					
32. GENERAL USE FOREIGN INTELLIGENCE NOT CONSIDERED									
33. KEYWORDS (Precede EACH with Security Classification Code) (U) Burn injury; (U) Topical therapy; (U) Sulfamylon; (U) Wound excision; (U) 5% Sulfamylon acetate solution; (U) Humans; (U) Autografts									
34. TECHNICAL OBJECTIVE, 35. APPROACH, 36. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.) 23. (U) The military relevance of improving burn wound care will be realized in increased troop survival following thermal injury. Newer methods under current investigation include the use of 5% aqueous Sulfamylon soaks, excision of eschar from burned soldiers, planned evaluation of cerium silver sulfadiazine, and the use of frozen homograft in burn wound care. Our objective is to further define the use of these methods. 24. (U) Patients admitted to the Institute of Surgical Research for care of thermal injuries receive burn wound care based on the specific injury. The 5% aqueous Sulfamylon soaks, excision of the eschar, and other modalities of wound care may be used. 25. (U) 7710 - 7809 Treatment with the Sulfamylon soaks was utilized in 126 patients, and 5 patients exhibited some form of allergic reaction. This 3.96% incidence of reactions noted was the only significant adverse reaction to this medication. These results support the continued use of 5% Sulfamylon soaks on a routine basis. Burn wound excision continued to be extensively utilized in an attempt to decrease the burn wound size and reduce the infectious complications. Tangential, full-thickness excision to viable fat, and excision to fascia were all utilized and are undergoing continued evaluation. Of the 73 patients (31% of total admissions) who underwent some type of operative excision in 1977, excision to fascia was performed in 24 patients (10%), escharectomy was performed in 27 patients (11.5%), and tangential excision of burn wounds of hands was performed in 19 patients (8%). Following studies of personnel utilization, cost of establishment, and clinical use, it was felt that a frozen homograft bank would not result in sufficient patient benefit, and therefore this project was discontinued. Also during this year the development of the new topical agent cerium silver sulfadiazine was stopped because of results of the long-term toxicity studies and product stabilization difficulties.									

* Available to contractors upon originator's approval

DD FORM 1498

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ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY:
5% AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL TREATMENT OF
BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

Theodore E. Plucinski, M.D., Lieutenant Colonel, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3SI61102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY:
5% AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL TREATMENT OF
BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

Investigators: Theodore E. Plucinski, M.D., LTC, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Improvement in care of the burn wound continues to be a major goal of the Institute of Surgical Research. Newer methods under current investigation includes the use of 5% aqueous Sulfamylon soaked dressings. The patients admitted to the Institute of Surgical Research for treatment of thermal injuries receive burn wound care based on the needs of the specific injury. Under appropriate conditions, 5% aqueous Sulfamylon soaked dressings are utilized. During the reporting period, a total of 126 patients required 5% Sulfamylon soaked dressings in the course of the care of their burned wounds. These dressings were employed both to the burn wounds and following skin grafting. A 3.9% incidence of significant skin rash was noted as the only significant adverse reaction due to this medication. These results support its continued use on a routine basis.

Burn injury
Topical therapy
5% Sulfamylon acetate solution
Humans

EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: 5% AQUEOUS
SULFAMYLCN SOAKS USED IN TOPICAL TREATMENT OF BURNED SOLDIERS

The evaluation of 5% Sulfamylon acetate solution as used in the topical treatment of burn wounds has been continued at this Institute. During the reporting period of 1 Oct 77 - 30 Sep 78, 234 patients were admitted to the U.S. Army Institute of Surgical Research. Of these 234, 126 had dressings employed for burn wound care that were wetted with 5% aqueous Sulfamylon solution. During this period, 256 split thickness skin autograft procedures were performed on 108 patients. Five percent aqueous Sulfamylon soaked dressings were used in conjunction with these skin grafting procedures in most patients. The Sulfamylon acetate soaked dressings are used either as continuous wet dressings in preparing the wound for skin grafting or as wet to dry dressings utilized to debride burn wounds. Following most grafting procedures wherever meshed grafts are applied, dressings soaked with 5% Sulfamylon acetate solution are applied in an attempt to decrease bacterial growth and protect the graft.

Occasional respiratory problems mainly in the form of hyperventilation have been noted in some patients who have had the application of Sulfamylon soaked dressings to extensive ungrafted burn wound. This hyperventilation uniformly resolves following discontinuance of the application of Sulfamylon solution to the dressings. Skin allergies to the sulfa solution continued to be noted with five such reactions recorded in 126 patients. This represents an incidence of 3.9% allergic skin reactions. In the five patients who developed allergic reactions where rapid resolution of the reaction did not occur following the administration of an antihistamine, the 5% Sulfamylon soaked dressings were discontinued. No other adverse reactions were noted in reviewing the clinical course and laboratory data of this group of patients.

The use of 5% Sulfamylon acetate soaked dressings continues to be an important component of the treatment of patients with burn wounds, both in the preparation of burn wounds for skin grafting and in the protection of meshed grafts once placed upon the wound. Its widespread use in severely burned patients with a low incidence of allergic reactions and absence of other side effects support its continued use in burn treatment.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN
INJURY: EXCISION OF ESCHAR IN BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

William F. McManus, M.D., Lieutenant Colonel, MC
Richard C. Treat, M.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN
INJURY: EXCISION OF ESCHAR IN BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

Investigators: William F. McManus, M.D., Lieutenant Colonel, MC
Richard C. Treat, M.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Improvement in the care of the burn wound continues to be a major goal of the US Army Institute of Surgical Research. Newer methods of treatment under current investigation include excision of eschar from burned soldiers. Patients admitted to the Institute of Surgical Research for treatment of thermal injuries receive burn wound care based upon specific injury needs. Under appropriate conditions, excision of eschar is utilized. Burn wound excision continues to be utilized in an attempt to decrease the burn wound size and to reduce septic complications. Tangential, full-thickness excision to viable fat and excision to the level of investing fascia continue to be evaluated.

Burn injury
Wound excision
Humans

EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY:
EXCISION OF ESCHAR IN BURNED SOLDIERS

No significant changes in patient selection, operative technique or results occurred in the use of excision as a form of burn wound therapy. Excision of the burn wound and subcutaneous tissue to the level of investing fascia, escharectomy, and tangential excision with immediate split-thickness autografting continue to be utilized at the US Army Institute of Surgical Research. Eighty-five patients (36% of admissions) underwent some type of excision during calendar year 1977.

Excision of burns to the layer of investing fascia was an operative procedure reserved primarily for patients with burns in the 40% to 60% total body surface range. Excision in burns larger than 60% has been shown to have no effect on the patient mortality in a review reported by Levine and others (1). Excision was not routinely used in burns of less than 40% of the total body surface; however, under certain circumstances, such as proven localized invasive burn wound infection, this type of therapy did prove to be successful.

Following excisional therapy, wound coverage with cutaneous autografts, allografts, or xenografts is performed. Coverage of fascia with dressings has uniformly resulted in fascial deterioration and an unacceptable result. The type of biologic material chosen for fascial coverage depends on the indications for the operative procedure, the appearance of the fascia following the excision, and the condition of the patient during the operative procedure. Routinely, 70% to 100% take of autograft can be expected if the fascia has been completely cleared of fat without undue tissue damage. When either allograft or xenograft is used as biologic covering for fascia, a bed of granulation tissue suitable for accepting autograft usually develops within 5 to 10 days. The development of a synthetic material which can be used to cover the fascia following excision, or the use of immunosuppression following excision which would allow the long-term use of allograft, may improve the usefulness of this technique of excision. Twenty-four patients underwent formal excision of their burn wounds at the US Army Institute of Surgical Research during the past year.

Excharectomy has been utilized both in the early and later post-burn course in burn wound care. In the early post burn period, this type of excision has been used primarily on small burn wounds. We have preferentially used excision to fascia on larger wounds because of less blood loss associated with an excision to fascia and a more acceptable take of autograft on fascia than on viable fat. Later in the post

1. Levine BA, Sirinek KR, Pruitt BA Jr: Wound excision to fascia in burn patients. Arch Surg 113:403-407, 1978.

burn course, this type of excision is used to remove tenacious eschar to achieve more rapid burn wound coverage with autograft. In both of these situations, excision of the entire eschar with a guarded knife down to freely bleeding fat, followed by application of cutaneous allografts, or xenografts, or sulfa soaks to the excised area, is most commonly performed. Dressings are changed every 2 days, and within 7 to 14 days, a lush bed of granulation tissue develops. Autografting can easily be undertaken in this situation. Blood loss may be massive during this procedure and may limit the extent of excision. The use of tourniquets on extremities undergoing escharectomy significantly reduces this blood loss. In the previous year, 15 patients underwent escharectomy as a form of treatment for their burn wounds.

Tangential excision as described by Janzekovic and Douglas Jackson (2) has been used at our unit primarily for the treatment of deep second degree burns of the hands. More superficial burns of the hands, which are characterized by a wet surface sensitive to pin-prick, may be treated in the standard fashion and expected to heal in less than three weeks with a satisfactory epithelial covering. Deeper second-degree burns, which are usually white, insensitive, and not as moist on the surface, will take longer to heal; but more importantly, they usually heal with unsatisfactory epithelium which is too thin to withstand minor trauma associated with normal activities of daily living and tends toward hypertrophic scarring. Such hands are best treated by tangential excision with immediate autograft placement.

Uniformly excellent results following tangential excision with immediate placement of autograft have been noted at our Institute. In addition, nearly normal return of function occurs much more rapidly with this form of treatment which significantly improves the patient's overall rehabilitation following thermal injury. Nineteen patients underwent this form of excisional therapy, and as in the past, one-third to one-half of these patients had both hands excised and grafted.

2. Jackson D, Stone PA: Tangential excision and grafting of burns: The method and report of 50 cases. Br J Plast Surg 4:416-426, 1972.

PRESENTATIONS AND/OR PUBLICATIONS

None

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN
INJURY -- ALKALINE PHOSPHATASE IN THE HEALING BURN
WOUND OF THE RAT

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

Gary H. Price, PhD, Major, MSC
John Dubois, BS
Charles S. Gilbert, SP-5

Reports Control Symbol MEDDH-288 (R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN
INJURY -- ALKALINE PHOSPHATASE IN THE HEALING BURN
WOUND OF THE RAT

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

Investigators: Gary H. Price, PhD, Major, MSC
John Dubois, BS
Charles S. Gilbert, SP-5

Reports Control Symbol MEDDH-288(R1)

Alkaline phosphatase extracted from the granulation tissue of the healing burn wound of the rat has been tested for its response to heat and urea inactivation, neuraminidase treatment, pH, and a variety of amino acids and other compounds known to affect its phosphomonoesterase activity. It is sensitive to chemical inhibition in a manner similar to isoenzymes from bone or liver, but its sensitivity to heat and urea inactivation falls between those of bone and liver isoenzymes. The possibility that it is composed of a mixture of bone and liver type isoenzymes cannot be ruled out by the means used in this study. Neuraminidase sensitivity indicates the presence of a terminal sialic acid residue on the polysaccharide portion of the enzyme. Optimum pH was determined to be 9.3 at the substrate concentration and in the buffer system employed. Levamisole was the most effective inhibitor tested. The inhibition by L-homoarginine was found to be pH dependent. The healing burn wound provides an excellent model for study of the nature and role of alkaline phosphatase in wound repair, due to the availability of relatively large amounts of enzyme.

Wound healing
Burns
Granulation tissue
Alkaline phosphatase

EVALUATION OF JRN WOUND CARE IN TROOPS
WITH BURN INJURY -- ALKALINE PHOSPHATASE IN THE
HEALING BURN WOUND OF THE RAT

Alkaline phosphatase (AP) (EC 3.1.3.1) has been studied in developing, healing, normal and neoplastic tissues from human and animal sources, generally demonstrating positive correlation with rate of healing, transport processes and tumor growth. Among the tissues in which the enzyme has been studied are included rat sponge-induced subcutaneous granulation tissue (1), and skin palatal gingiva and tongues of rats during healing after experimental wounding (2). This paper presents a study of AP extracted from granulation tissue of rats which had been subjected to a standardized burn (3). In an effort to characterize the enzyme from granulation tissue and, if possible, to distinguish it from AP isoenzymes extracted from liver, bone and intestine, a variety of inhibitors were employed, heat lability and neuraminidase sensitivity measured and pH optimum and electrophoretic mobility on polyacrylamide gels determined. The following presents our findings.

MATERIALS AND METHODS

Adult male Sprague-Dawley (Holtzman strain) rats were anesthetized with pentobarbital administered intraperitoneally (1 mg/25 g body weight), sheared of hair over the back and belly and placed in a device to limit and control burn size. The exposed dorsal and ventral areas were then immersed in boiling water for 10 seconds on the dorsum and 2.5 seconds on the abdomen, producing a full thickness burn over 50% of the body surface. On the twentieth day after burning, the rats were killed by dry ice anesthesia followed by heart puncture and exsanguination. Livers were blanched by perfusion with normal saline. Pieces of rib bone were scraped clean of soft tissue. Sections of the small intestine in the region of the jejunum were excised and flushed of contents, and the kidneys removed. The burn eschar was stripped from the granulation tissue. Tissue slices of normal skin were prepared by stripping the fascia and removal of fibrous connective tissue and excision of the deep dermal layer using a Stadie-Riggs tissue slicer. Each tissue was then placed in approximately 1 ml of water/100 mg of tissue and homogenized at 26,000 rpm in a Tissumizer (Tekmar Company, Cincinnati, Ohio). The homogenates were added to 2 volumes of n-butanol (4) and mixed on a Vortex mixer at maximum speed for one minute. The phases were separated by centrifugation at 4°C for 10 minutes in a Sorvall Model RC-2B. The

1. Vizioli MR, Bozzo L & Valdrighi L: Alkaline phosphatase activity and the development of rat sponge induced granulation tissue. *Acta Anat* 83: 60-69, 1972.
2. Carranza FA, Jr & Cabrini RL: Histo enzymic behavior of healing wounds. *J Invest Dermatol* 40: 27-36, 1965.
3. Walker HL & Mason AD, Jr: A standard animal burn. *J Trauma* 8: 1049-1051, 1968.
4. Morton RK: The purification of alkaline phosphatases of animal tissues. *Biochem J* 57: 595-603, 1954.

aqueous phases were exhaustively dialyzed against three changes of buffer, one liter of 0.025 M Tris, pH 7.5 at 4°C.

AP was assayed by a modification of the method of Bessey, *et al* (5), using 0.5 ml of 2-methyl-2-amino-1-propanol (AMP) buffer (Sigma 325-3), 0.75 M, pH 9.4 at 37°C, containing 1 mM MgCl₂. The final reaction volume of 2 ml included 0.5 ml of 15.2 mM p-nitrophenyl phosphate (Sigma 104). The reaction was stopped by addition of 3 ml of 1 N NaOH and the absorbance read at 410 nanometers using a Gilford 240 spectrophotometer with 1 cm light path.

Inhibition studies were carried out by addition of aqueous solutions of the inhibitor to the assay mixture prior to the addition of substrate. Inhibitor concentrations were calculated with respect to final assay mixture. Duplicate assays were set up using the highest concentration of each inhibitor, and the pH of the final assay mixture measured. In three experiments, AP from rat bone, liver and granulation tissue was pre-incubated with urea in concentrations ranging from 2 to 4 molar for 9 minutes at 37°C, in 1 M diethanolamine buffer, pH 9.6. Urea was added to the reaction mixture to bring the final concentration of all to 4 molar at the time of addition of substrate to start the reaction. Measurement of pH optimum was performed in 0.75 M AMP buffers prepared in the range of pH 8.4 to 10.4 at 37°C.

Heat lability was determined by heating the assay mixture containing the enzyme and buffer in an oil bath at 56°C. After the prescribed period of incubation, each sample was placed in an ice bath for a few minutes, allowed to return to room temperature, substrate added and the assay carried out as usual. Neuraminidase sensitivity was measured by incubating the AP with *Cl. perfringens* neuraminidase. Type VIII (Ec 3.21.18, Sigma N-5631) for 16 hours at pH 7.2, 37°C (6). Electrophoretic mobility was determined by polyacrylamide gel electrophoresis on 7% gels (7,8). AP bands were visualized by incubating the gels in a histochemical stain composed of 100 ml of a 0.15 M Tris buffer, pH 10.3, containing 1.0 mM MgCl₂ and 200 mg each of alpha-naphthyl phosphate and Fast Blue BB diazonium dye (Dajac Labs, Haven Chemical, Philadelphia, PA). All reagents used were reagent grade. Amino acids were purchased from Pierce, Box 117, Rockford, IL, except for L-arginine (Aldrich Chemical Co., Inc, Milwaukee, WI) and D-cysteine (Sigma). Levamisole was procured from Scott-Pittman, Washington Cross, NJ.

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RESULTS

The phosphomonoesterase activity of the enzyme extract was determined to be linear with respect to sample size and time of incubation. The pH optimum curve, activity vs. pH, appears in figure 1. The rate of hydrolysis of p-nitrophenylphosphate at a final concentration of 3.8 mM in 0.75 M AMP buffer is found to be greatest at pH 9.33 at 37°C.

The heat labilities of rat granulation tissue alkaline phosphatase (RGTAP) and the isoenzymes of liver and bone, expressed as percent loss of activity vs. incubation time at 56°C, are shown in figure 2. The time required for loss of RGTAP 50% activity was 7.5 minutes. The bone enzyme was the most labile, losing 50% activity at 5 minutes, and that from liver the most stable, requiring 9 minutes at 56°C for loss of half its activity. Treatment with neuraminidase caused a 40% decrease in mobility on polyacrylamide gel electrophoresis (PAGE). Several samples of RGTAP demonstrated three bands on PAGE, with electrophoretic mobilities similar to those observed in two kidney samples.

The inhibition of AP from granulation tissue, bone and intestine by L-histidine and L-homoarginine are depicted in figures 3 and 4 respectively. L-histidine inhibited the three isoenzymes to the same extent; L-homoarginine was a much more effective inhibitor of bone and granulation tissue enzymes than of intestinal AP. For all three isoenzymes there was an increase in inhibition as the pH was increased from 9.6 to 10.3. Bone and granulation tissue isoenzymes responded to L-homoarginine in exactly the same manner. Although we did not test the effect of L-homoarginine on rat liver AP, it has been reported that human liver and bone isoenzymes are inhibited by this amino acid in a similar manner (9). Inhibition of granulation tissue, bone, intestine and liver AP by L-phenylalanine is shown in figure 5. Intestinal AP was inhibited to a greater extent by this amino acid than the other three isoenzymes. Inhibition of the granulation tissue, bone and liver enzymes by urea is shown in figure 6. In two of three experiments, using diethanolamine buffer, the slopes of the lines representing relative residual activity vs. concentration of urea during pre-incubation were not significantly different and covariance analysis indicated that liver and granulation tissue enzymes were inactivated to the same extent, significantly less than bone enzyme ($p < 0.05$). In the third experiment, the slopes differed significantly, precluding similar analysis. Similar inhibitory effects of L-tryptophan and imidazole towards RGTAP are shown in figure 7. Levamisole shows very strong inhibition at pH 9.6 and 10.3 (Fig. 8). None of the inhibitors tested caused any measurable change in pH under these experimental conditions. The effects on RGTAP of 23 amino acids and 4 selected drugs, as well as zinc (II) and urea at single concentrations are listed in Table 1. Ouabain, an inhibitor of some membrane bound enzymes, was found to have no inhibitory effect at concentrations ranging from 10^{-6} to 10^{-3} molar. The specific activity of AP in dialyzed butanol extracts of granulation

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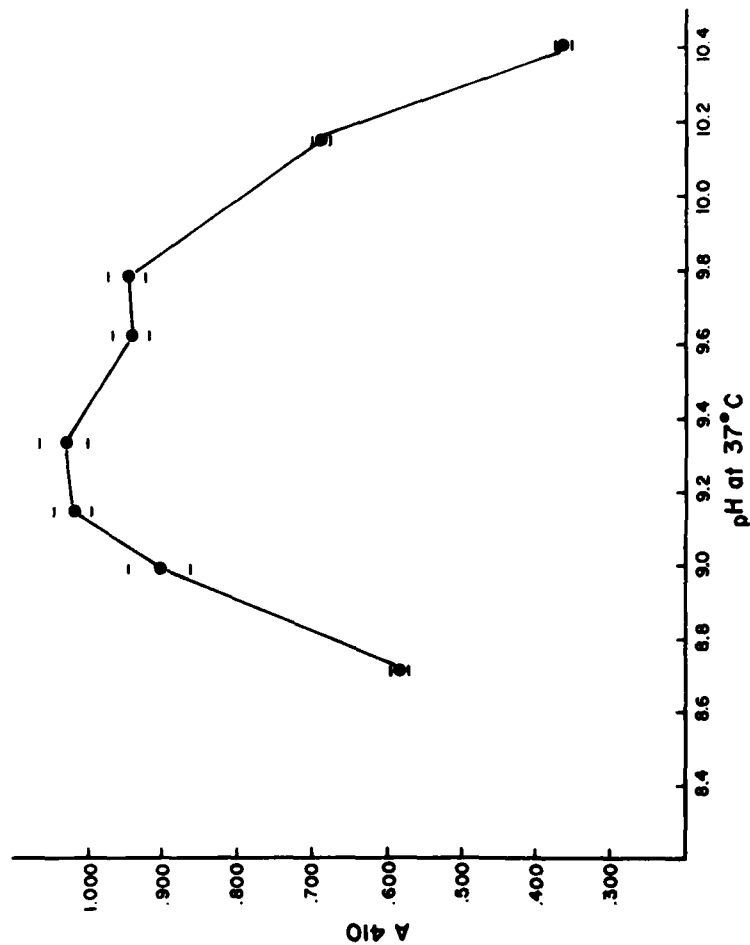


Fig. 1. Effect of pH on rat granulation tissue alkaline phosphatase (RGTAP) - Points represent the mean (\pm S.D.) of triplicate measurements of the phosphomonoesterase activity of a butanol extract of homogenized granulation tissue excised from the healing wound of a rat 20 days after burning. The shoulder on the curve was observed in other experiments performed on similar specimens.

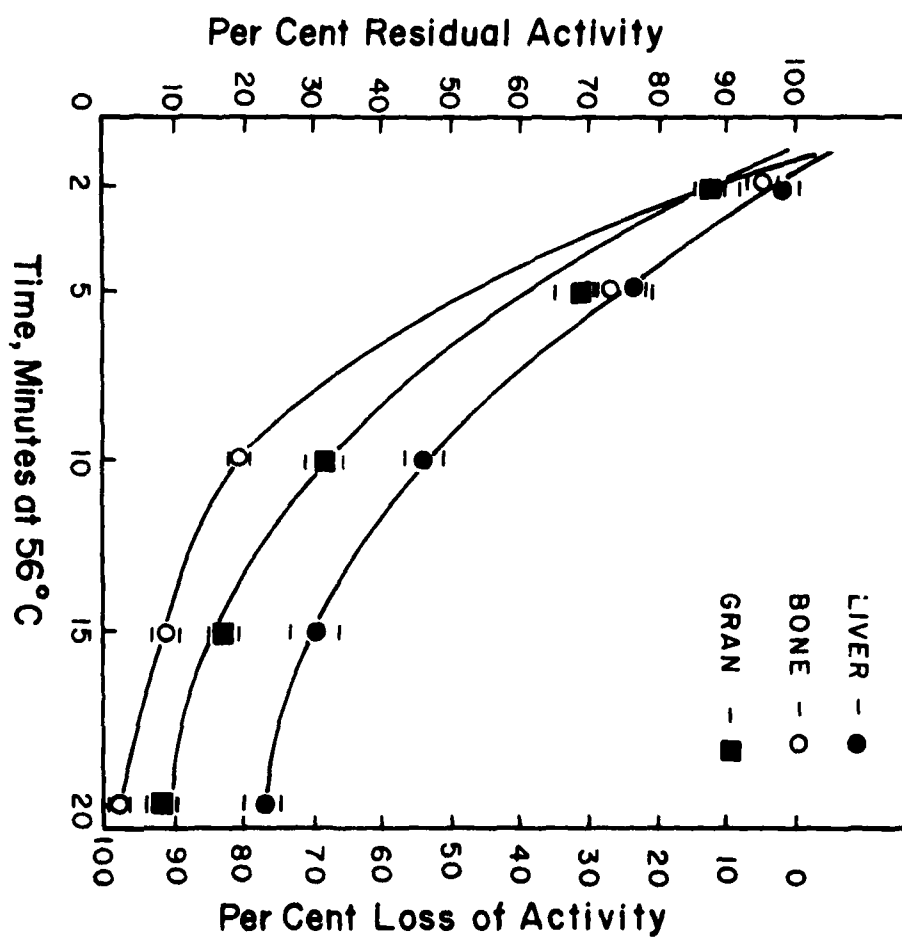


Fig. 2. Effect of heating on activity of RGTAP, bone and liver alkaline phosphatase - Points represent the mean (\pm S.D.) of triplicate measurements of phosphomonoesterase activity of butanol extracts of homogenates of rat bone, liver and granulation tissue after incubation in AMP buffer at 56°C for 0, 2, 5, 10, 15 or 20 minutes. The unheated enzymes represent 100% residual activity.

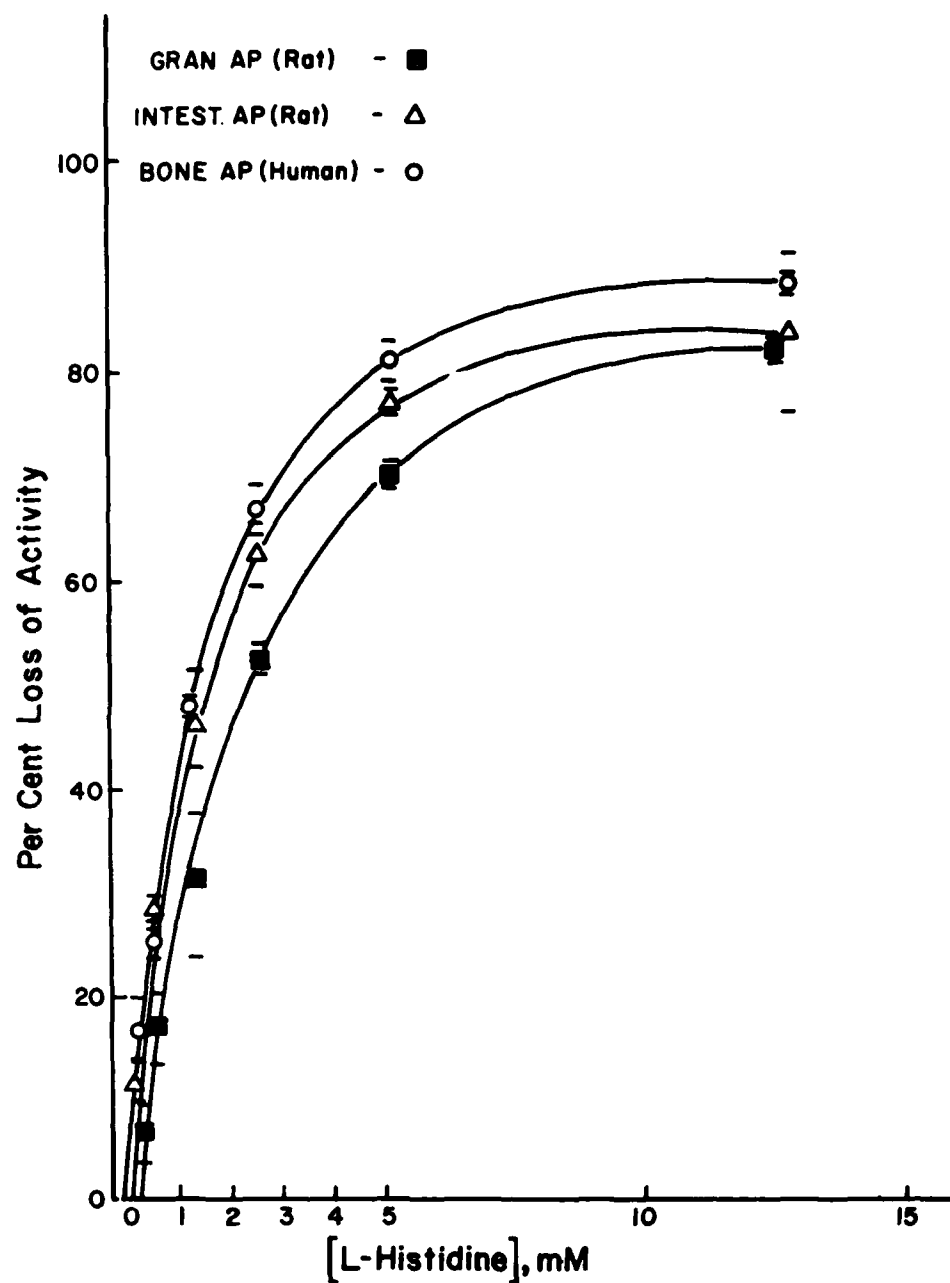


Fig. 3. Effect of L-histidine on activity of bone, liver and granulation tissue alkaline phosphatase - Points represent the mean (\pm S.D.) of triplicate measurements of phosphomonoesterase activity of butanol extracts of homogenates of bone, liver and granulation tissue in the presence of L-histidine. Data points are expressed as per cent activity in the absence of L-histidine.

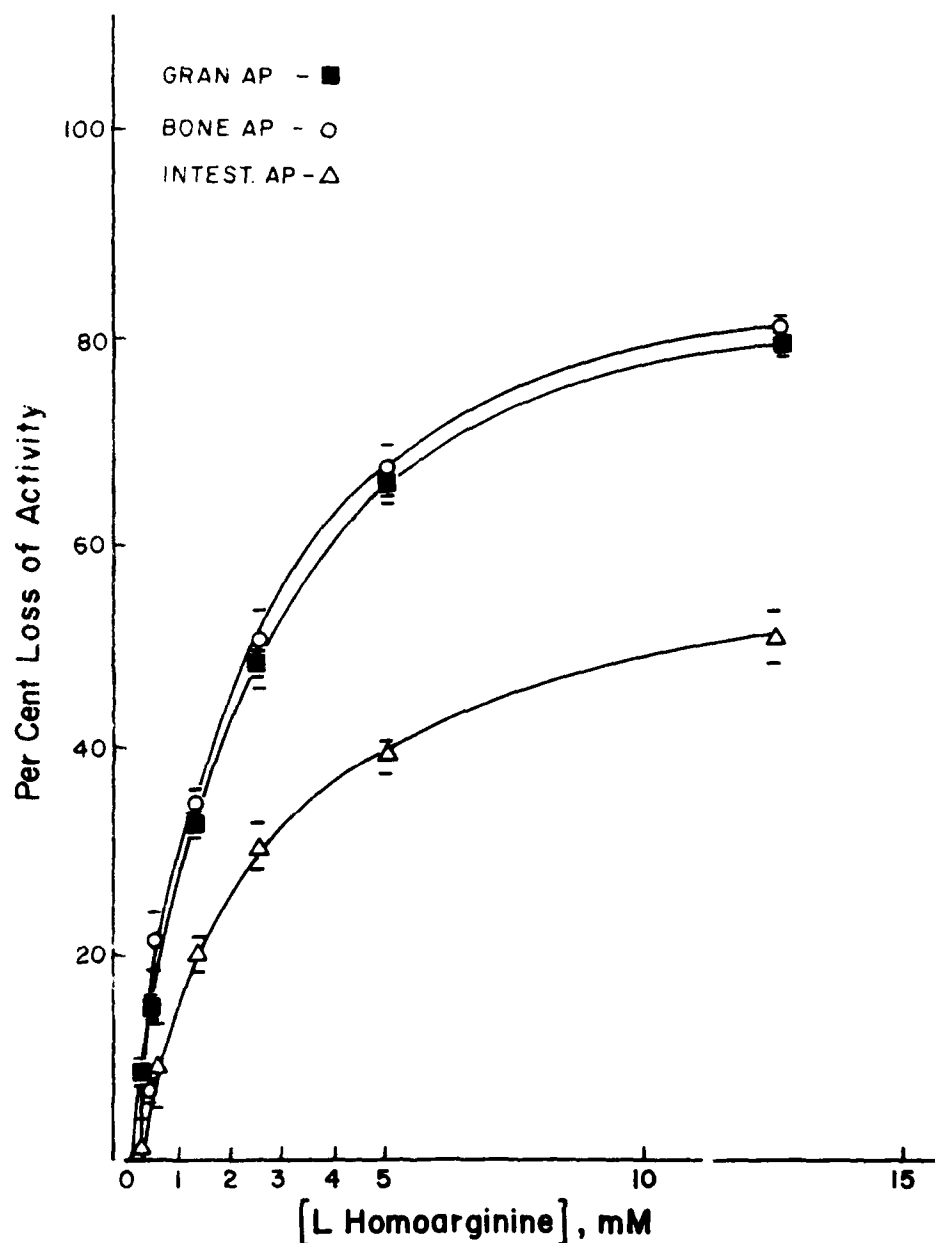


Fig 4a. Effect of L-homoarginine on activity of bone, intestine and granulation tissue alkaline phosphatase at pH 9.6 - Points represent the mean (\pm S.D.) of triplicate determinations of phosphomonoesterase activity of butanol extracts of homogenates of rat bone, intestine and granulation tissue in the presence of L-homoarginine at pH 9.6. Data points are expressed as per cent of activity in the absence of L-homoarginine.

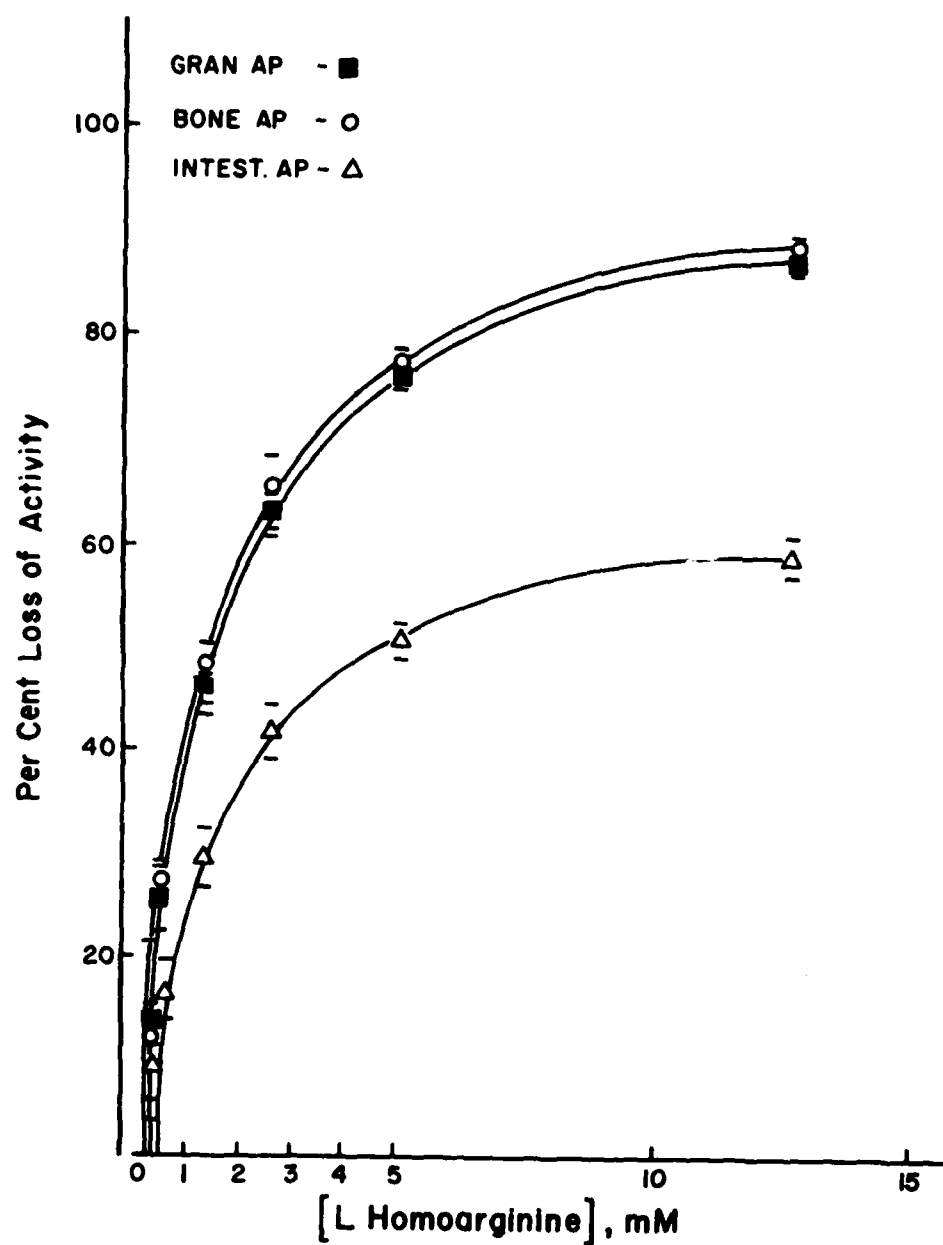


Fig. 4b. Effect of L-homoarginine on activity of rat bone, intestine and granulation tissue alkaline phosphatase at pH 10.3 - Experimental conditions are the same as those shown in Fig. 4a except for change from pH 9.6 to pH 10.3.

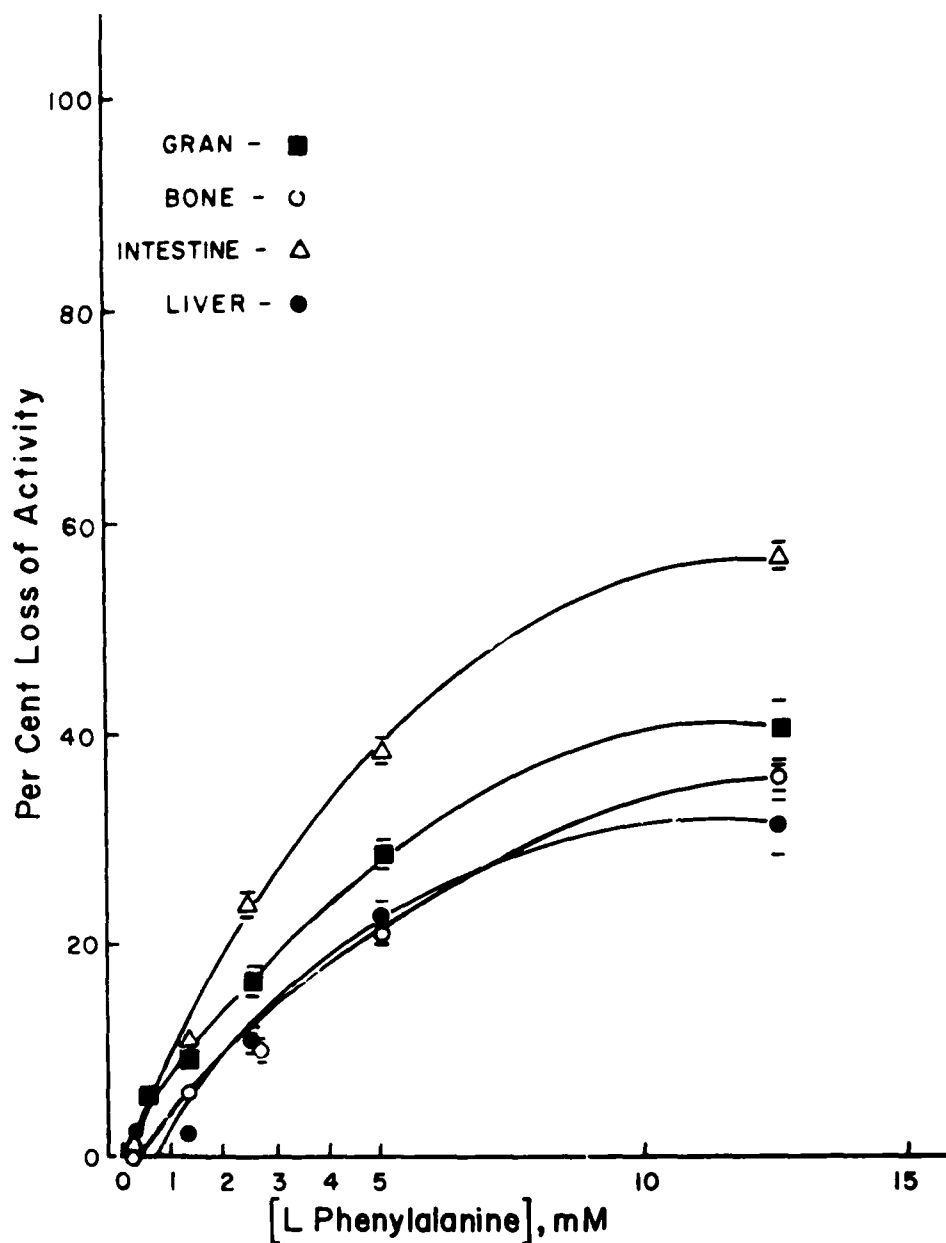


Fig. 5. Effect of L-phenylalanine on activity of rat bone, liver, intestine and granulation tissue alkaline phosphatase - Points represent the mean (\pm S.D.) of triplicate determinations of phosphomonoesterase activity of butanol extracts from homogenates of rat bone, liver, intestine and granulation tissue in the presence of L-phenylalanine, expressed as per cent of activity in the absence of L-phenylalanine.

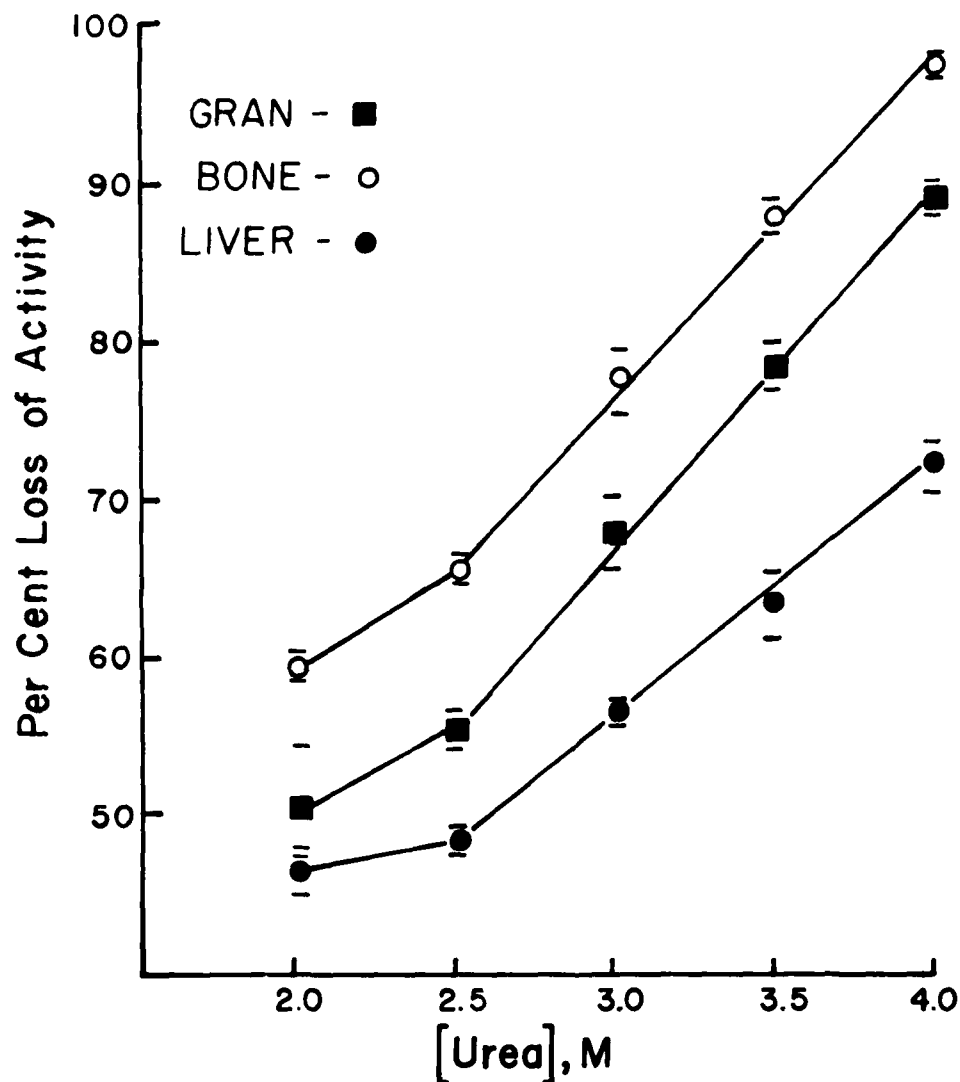


Fig. 6. Effect of incubation in urea on activity of rat bone, liver and granulation tissue alkaline phosphatase - Points represent the mean (+ S.D.) of triplicate measurements of residual per cent phosphomonoesterase activity of butanol extracts of homogenates of rat bone, liver and granulation tissue after pre-incubation in urea. Extracts were incubated in diethanolamine buffer at 37°C for 9 minutes, substrate was added, and assay carried out for 5 minutes.

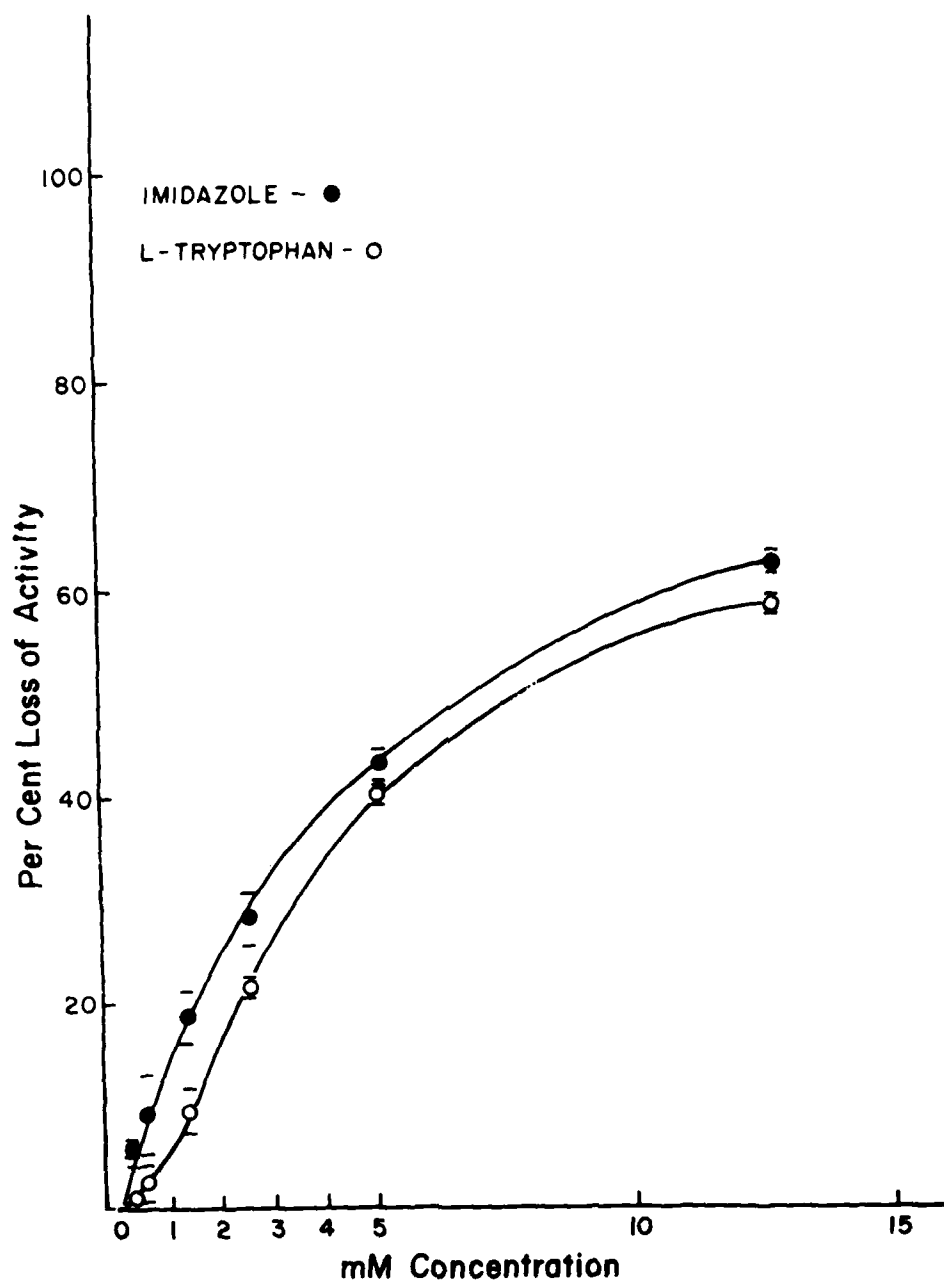


Fig. 7. Effect of imidazole and L-tryptophan on activity of rat granulation tissue alkaline phosphatase - Points represent the mean (+ S.D.) of triplicate measurements of phosphomonoesterase activity of rat granulation tissue alkaline phosphatase in the presence of imidazole or L-tryptophan, expressed as per cent of activity based on the activity in the absence of inhibitors.

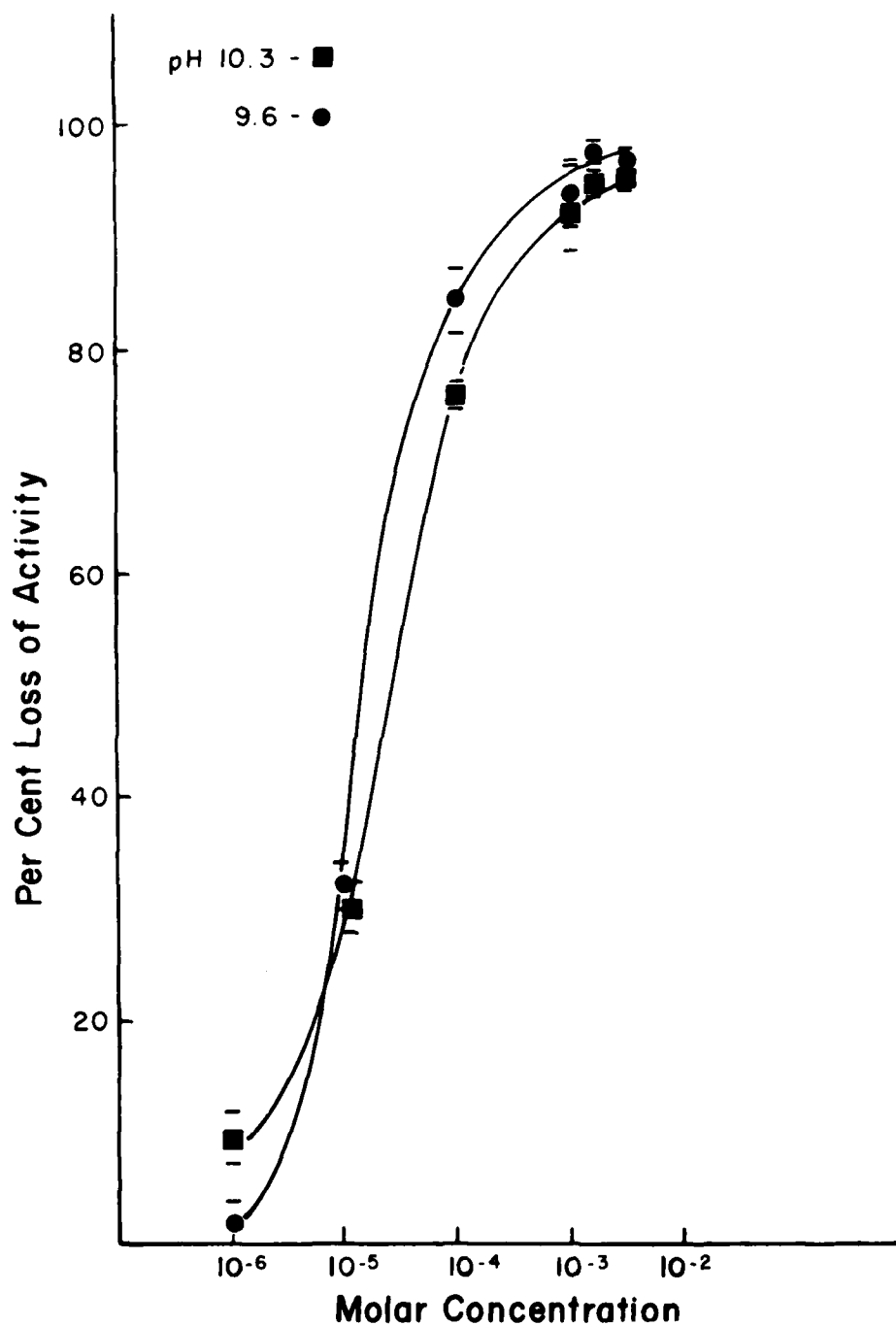


Fig. 8. Effect of Levamisole on the activity of rat granulation tissue alkaline phosphatase at pH 9.6 and pH 10.3 - Points represent the mean (+ S.D.) of triplicate determinations of phosphomonoesterase activity of rat granulation tissue alkaline phosphatase in the presence of Levamisole at pH 9.6 or pH 10.3, expressed as per cent loss of activity, compared with activity in the absence of Levamisole. 173

Table I
Rat Granulation Tissue Alkaline Phosphatase Response to
Amino Acids and Selected Drugs

Compound	Final Concentration (mM) in Assay	% Inhibition
L-Alanine	12.50	14.0
L-Arginine	12.50	49.9
L- β -Asparagine	12.50	10.1
L-Aspartic Acid	*	4.4
D-Cysteine	12.50	92.5
L-Cystine	*	2.1
L-Glutamic Acid	*	21.6
L-Glutamine	12.50	14.6
Glycine	12.50	38.5
L-Histidine	12.50	83.0
L-Homoarginine	12.50	84.0
L-Hydroxyproline	12.50	13.2
L-Isoleucine	12.50	41.5
L-Leucine	12.50	44.8
L-Lysine	12.50	25.0
L-Methionine	12.50	38.3
L-Phenylalanine	12.50	48.2
L-Proline	12.50	2.1
L-Serine	12.50	18.3
L-Threonine	12.50	29.5
L-Tryptophan	12.50	59.0
L-Tyrosine	*	0.1
L-Valine	12.50	48.0
Epinephrine	*	5.7
Histamine	12.50	45.4
Imidazole	12.50	64.0
Levamisole	5.0	96.5
Urea	2000	83.8
Zinc (Zn++)	1.0	91.0

* Saturated at 22°C.

tissue and normal skin was found to be 2.13 ± 2.01 IU/mg protein and $1.06 \pm 0.8 \times 10^{-3}$ IU/mg protein, respectively.

DISCUSSION

Although AP activity is very low in mature normal skin, it is relatively abundant in both developing normal skin and in regenerating skin following a wound. Such activity has been demonstrated by histochemical techniques to occur in the capillaries, subepidermal capillary plexus and mast cells of

embryonic normal skin at the fourteenth day with more intense activity in capillaries in the subdermal plexus at the eighteenth day and in dermal papillae and hair bulbs in the newborn rat (10). Enzyme activity in rat polymorphonucleocytes (PMN) has been described (11). AP has been reported to occur in PMN's, fibroblasts and chondrocytes during the formation of hemopoietic ossicles induced by implantation of demineralized bone-matrix collagen in the rat (12). The peak enzyme activity during the healing of experimental incised wounds and burns was concurrent with the maximum rate of collagen formation (13).

In granulation tissue induced by subcutaneous implantation of polyvinylchloride sponges in rats, activity was observed in the cytoplasm of very active migratory cells and fibroblasts on the fifth day and in the edges of forming tissue on the walls of newly developing capillaries on the seventh day. By the seventeenth day, the entire granulation tissue, including collagen fibers, tissue cells and capillary walls, demonstrated strong enzyme activity (1). In healing wounds of palatal gingiva of rats it was shown that the enzyme activity at the third day postinjury was largely confined to the PMN's and some monocytes in the inflammatory exudate and to the vessels of the corium. At the seventeenth day, intense activity was demonstrated in proliferating fibroblasts, some in inflammatory cells and in newly formed capillaries. By the end of the third week activity was reduced (2).

The events of fibroblastic and leukocytic infiltration, capillary formation and collagen synthesis following burn trauma have been well documented and recently reviewed (14). As expected, the granulation tissue formed in the healing burn wound of the rat about the third week postburn demonstrates a relative abundance of AP activity; there remain, however, questions about the physiologic role of the enzyme and its responses to various physical and chemical stimuli or inhibitors, as well as its immunologic relationships to isoenzymes from other tissues, mechanisms of regulation of synthesis and specific activity.

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Evidence for functional significance may be approached by first determining which isoenzyme is most similar to that or those found in granulation tissue, and studying in more detail the common histologic and biochemical events shared by the two types of tissue in developmental stages. The burn wound offers a striking advantage over the incised wound as a model for the study of AP and its role in healing. The greatly increased amount of tissue involved serves as a source of sufficient enzyme to make practicable isolation and purification of the enzyme, as well as kinetic inhibition studies.

Isoenzymes from bone or liver can be distinguished from those of intestine, placenta or tumors by their thermolability (15). The AP from rat granulation tissue falls in the thermolabile group with bone and liver isoenzymes. The sensitivity of RGTAP to treatment with neuraminidase is similar to that observed in liver and bone (16) and distinguishes it from that of intestine which is not affected (6); RGTAP responds to L-homoarginine in a manner similar to the isoenzymes of bone and liver, unlike that of intestine (9). In contrast to Lin and Fishman's findings, we observed a greater inhibition at pH 10.3 than pH 9.6, possibly reflecting rat strain or tissue type differences. Treatment of these four isoenzymes with L-phenylalanine similarly groups RGTAP with bone and liver isoenzymes. The degree of inhibition of RGTAP by L-tryptophan is in close agreement with that reported for rat liver and bone (17) isoenzymes. Levamisole is a powerful uncompetitive inhibitor of AP; in studies carried out on extracts from canine tissues, it is unusual in that it inhibits the placental isoenzyme to the same extent as that from liver, bone, kidney and tumor. Only the isoenzyme from intestine can be differentiated using this inhibitor (18); however, the same author found that human bone, liver, kidney and spleen are highly sensitive to Levamisole, but placental and intestinal isoenzymes are relatively resistant (19). Our RGTAP inhibition curve can be superimposed on his canine liver-bone group curve.

On the basis of data from these studies, it is apparent the AP in rat

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burn wound granulation tissue most closely resembles that derived from bone or liver. Several authors have reported differential inhibition of bone and liver isoenzymes by urea treatment (20,21). In the Gorman and Statland studies, the samples used were sera, categorized as being of bone or liver origin based on clinical diagnosis, and the assay performed in AMP buffer; in Gerhardt's study, tissue extracts were used and the assay carried out in diethanolamine buffer. Using AMP buffer and human tissue extracts, we were unable to distinguish bone and liver isoenzymes under a variety of conditions, e.g., different urea concentrations and time of pre-incubation. In the urea pre-incubation experiments performed in diethanolamine buffer, it was possible to distinguish bone from liver AP, within each experiment. Variation among experiments was sufficiently large that it would be impossible to predict the tissue source of an individual observation taken at random and compared with any one of the sets of curves.

The only clearcut conclusion from these data is that bone isoenzyme is more sensitive to urea denaturation than is liver isoenzyme. This difference is similar in extent and direction to that observed in heat denaturation. Two effects of urea on APs, a reversible instantaneous inhibition and an irreversible inactivation which is tissue specific in degree, have been described (22). These experiments do not suggest whether the RGTAP is a distinct isoenzyme or a mixture of bone and liver types. Inasmuch as several cell types, e.g., leukocytes, fibroblasts and capillary wall epithelium demonstrate activity, the latter possibility should be considered.

PRESENTATIONS: None

PUBLICATIONS:

Price GH, Dubois J & Gilbert CS: Alkaline phosphatase in the healing burn wound of the rat. Submitted to Arch Biochem Biophys.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN
INJURY -- SULFONAMIDE INHIBITION OF HUMAN ALKALINE
PHOSPHATASE

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigator:

Gary H. Price, PhD, Major, MSC

Report Control Symbol MEDDH-288 (R1)

Unclassified

ABSTRACT

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REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH
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US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

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Sulfamylon is employed as an antibacterial agent in the treatment of severe burns. It is known to inhibit carbonic anhydrase, a zinc dependent enzyme; we have found that another zinc bearing enzyme, alkaline phosphatase (AP) is also strongly inhibited by sulfamylon as well as by other antibacterial or diuretic sulfonamides. Sulfamylon inhibits AP by a mixed mechanism, the noncompetitive component being greater than the competitive. The sulfonamide diuretic, furosemide, inhibits AP in a similar manner but the competitive contribution is greater. Methylation of the sulfonamide nitrogen changes the mechanism of inhibition to uncompetitive. AP from placenta is much less sensitive to sulfonamides than bone, liver, kidney, intestine or granulation tissue isoenzymes.

Burn injury
Humans
Alkaline phosphatase
Sulfamylon

EVALUATION OF BURN WOUND CARE IN TROOPS WITH
BURN INJURY — SULFONAMIDE INHIBITION OF
HUMAN ALKALINE PHOSPHATASE

Sulfamylon (mafenide acetate, homosulfanilamide acetate) is employed as an antibacterial agent in the treatment of severe burns. A substituted sulfonamide, it is an effective carbonic anhydrase inhibitor (1); its mode of inhibition is considered to be interaction with the zinc-bearing active site of the enzyme (2). Another enzyme whose activity is believed to be dependent upon zinc in the active site is alkaline phosphatase (AP) (3). We have demonstrated that a relative abundance of this enzyme is present in granulation tissue during healing of large surface burn wounds (4). Although the physiologic significance of AP, either in a normal or pathologic context, has not been well established, it seemed reasonable to test the effects of topical agents on the activity of granulation tissue AP. Several other sulfonamides are commonly used as diuretics, including acetazolamide and furosemide. Due to the structural similarities between sulfamylon and the sulfonamide diuretics, because AP is present in the kidney and because the methylated xanthine diuretic, theophylline, has been found to inhibit AP (5,6), acetazolamide and furosemide were also tested as AP inhibitors. The effects of inhibitors on AP isoenzymes from several tissue sources have been widely used as means to distinguish or categorize them. This study reports the effects of sulfamylon and acetazolamide on isoenzymes from bone, liver, kidney, burn wound granulation tissue, intestine and placenta, and the effects of several other sulfonamides on bone AP.

METHODS

Specimens of human liver, kidney, intestine and vertebral body bone were collected at necropsy from patients who had succumbed to severe thermal injury. A placenta was obtained following a normal live birth. Pieces of tissue weighing 1 to 10 grams were minced with scissors and added to 2-5 ml of 0.05 M phosphate buffer, pH 6.9, containing 0.1 M NaCl and 0.04% sodium azide, per gram of wet tissue. The tissues were homogenized (Tissumizer, Tekmar Corp., Cincinnati, Ohio) at 15,000 rpm for approximately one minute. Extraction with n-butanol (7), 2 ml/ml homogenate was done by shaking vigorously

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for 3 to 5 minutes in 50 ml polypropylene conical centrifuge tubes. The tubes were centrifuged at 3,000 rpm (International Model EXD) for 10 minutes, in a number 250 head. The aqueous layers were aspirated and re-extracted with 2 volumes of butanol. After separation from the lipid phase, the aqueous layer was centrifuged for 10 minutes at 26,000 $\times g$ in a Sorvall RC2B refrigerated centrifuge. The extracts were dialyzed at 4°C against three changes of 0.010 M Tris HCl buffer, pH 7.5. AP activity was assayed by a modification of the method of Bessey, et al (8), using 0.5 ml of a 2-methyl-1-amino-1-propanol buffer (AMP, Sigma 325-3), 0.75 M, pH 9.4 at 37°C, containing 1mM MgCl₂. The final reaction volume of 2 ml included 0.5 ml of 15.2 mM p-nitrophenyl phosphate (Sigma 104), except in the double-reciprocal experiments, in which the final concentrations of substrate were 8, 4, 2, 1, 0.5 and 0.25 mM. The enzyme extract was added last to start the reaction. The reaction was stopped by addition of 3 ml of 1N NaOH and the absorbance read at 410 nanometers. Aqueous solutions of the various inhibitors were added to the assay mixture prior to the addition of substrate. Inhibitor concentrations refer to the final assay volume prior to addition of NaOH.

Mafenide acetate (alpha-amino-p-toluene sulfonamide acetate) was obtained from Winthrop Laboratories, Division of Sterling Drug, Inc., NY 10016; N-methyl-p- (alpha-amino) toluenesulfonamide acetate (ISR-3) was synthesized at the Institute of Surgical Research (9). Acetazolamide was procured from Lederle Laboratories, Pear River, NY. Furosemide (Lasix) was from Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876; p- (n-methyl) (alpha-amino) toluenesulfonamide hydrochloride was obtained from E.R. Squibb & Sons. Para-carboxybenzenesulfonamide was purchased from ICN Life Sciences Group (K&K), Plainview, NY 11803. All other chemicals were reagent grade.

RESULTS AND DISCUSSION

Early investigators reached the conclusion that the inhibition of carbonic anhydrase by sulfonamides was of the non-competitive type (10,11), although later studies, using stopped-flow techniques, provided data from which Kernohan concluded that the mechanism was competitive (12). Under our experimental conditions, data were obtained which, visualized as the Lineweaver-Burk double reciprocal plot, $1/V$ vs $1/S$, at several concentrations of sulfamylon (Fig. 1), are characteristic of mixed inhibition, that is, having both

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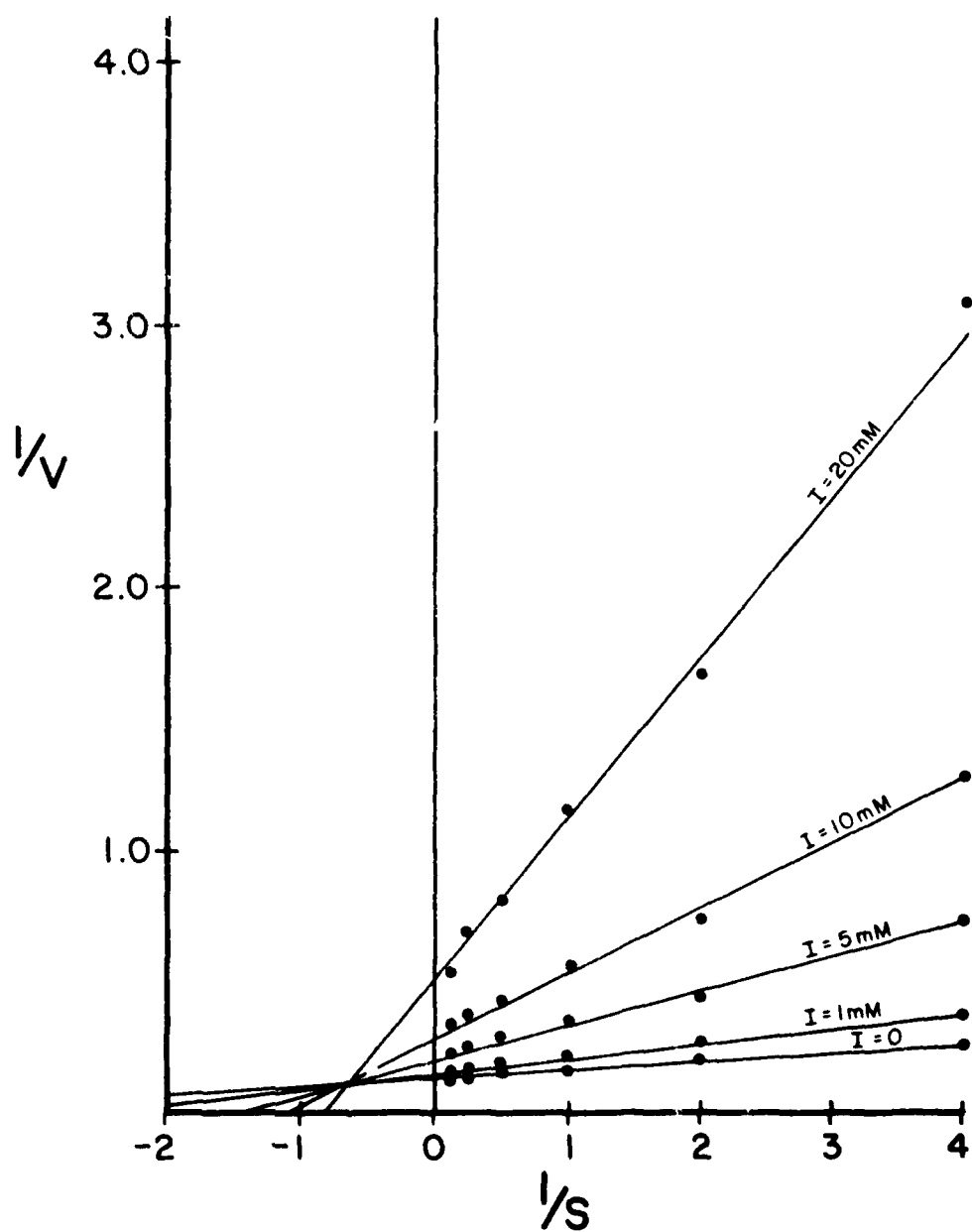


Fig. 1. Lineweaver-Burk plot, ($1/\text{absorbance}$ vs. $1/\text{millimoles-liter}^{-1}$) of sulfamylon inhibition of human bone alkaline phosphatase.

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ANNUAL RESEARCH PROGRESS REPORT. 1 OCTOBER 1977-30 SEPTEMBER 19--ETC(1)
SEP 78

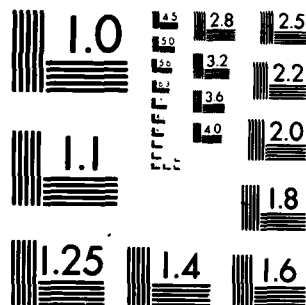
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MICROCOPY RESOLUTION TEST CHART
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competitive and noncompetitive characteristics. Pure noncompetitive inhibition, in which inhibitor binds to the enzyme directly in such a way as to prevent the breakdown of the subsequently formed active complex, yields a double reciprocal plot in which the lines intersect on the abscissa to the left of the ordinate. Pure competitive inhibition, in which case the inhibitor binds directly to the enzyme, retarding the rate of formation of the enzyme-substrate complex, yields a double reciprocal plot in which the lines intersect at a common point on the ordinate. Furosemide, despite a lack of similarity to sulfamylon in structure, displays remarkably similar behavior both in terms of effectiveness of inhibition and the characteristics of the Lineweaver-Burk plot, except that the intersection of the lines lies relatively closer to the vertical axis in the case of furosemide, whereas in the sulfamylon plot it is closer to the horizontal axis. This may be interpreted to mean that the competitive contribution is relatively greater for furosemide, and that noncompetitive characteristics predominate in sulfamylon inhibition (13). On the other hand, p-(N-methyl) (alpha-amino) toluenesulfonamide hydrochloride (SQ-684), which differs from sulfamylon only in the methylation of the sulfonamide nitrogen, and the counter ion, displays a totally different mechanism of inhibition. The double reciprocal plot (Fig. 2) shows a series of parallel lines characteristic of uncompetitive inhibition, in which the inhibitor binds only the enzyme-substrate complex, blocking further reaction and release of the normal product. The same type of inhibition has been attributed to the amino acids, L-homoarginine, L-phenylalanine, L-tryptophan and L-leucine, as well as Levamisole and imidazole, which have been used extensively in efforts to distinguish AP isoenzymes from different tissue sources (14,15).

Numerous reports have been published showing similar response by bone and liver AP isoenzymes to inhibitors. Rat granulation tissue AP has been recently shown to be similar to these isoenzymes (4) and this study indicates that sulfamylon is equally effective as an inhibitor of bone, liver, granulation tissue and kidney AP isoenzymes and only slightly less potent toward intestinal AP. The placental isoenzyme is markedly less sensitive to sulfamylon (Table 1). Acetazolamide inhibits liver, bone, granulation tissue, kidney and intestine isoenzymes to the same extent at several concentrations; the placental isoenzyme loses about one-third as much activity as the others.

13. Webb JL: Enzymes and Metabolic Inhibitors. vol.1, pp 160-162, Academic Press, NY. 1963.

14. Lin C-W, Fishman WH: L-homoarginine: An organ-specific, uncompetitive inhibitor of human liver and bone alkaline phosphohydrolases. J Biol Chem 247: 3082-3087, 1972.

15. Fishman WH: Perspectives on alkaline phosphatase isoenzymes. Amer J Med 56: 617-650, 1974.

4. Price GH, Dubois J, Gilbert CS: Alkaline phosphatase in the healing burn wound of the rat. (Submitted for publication).

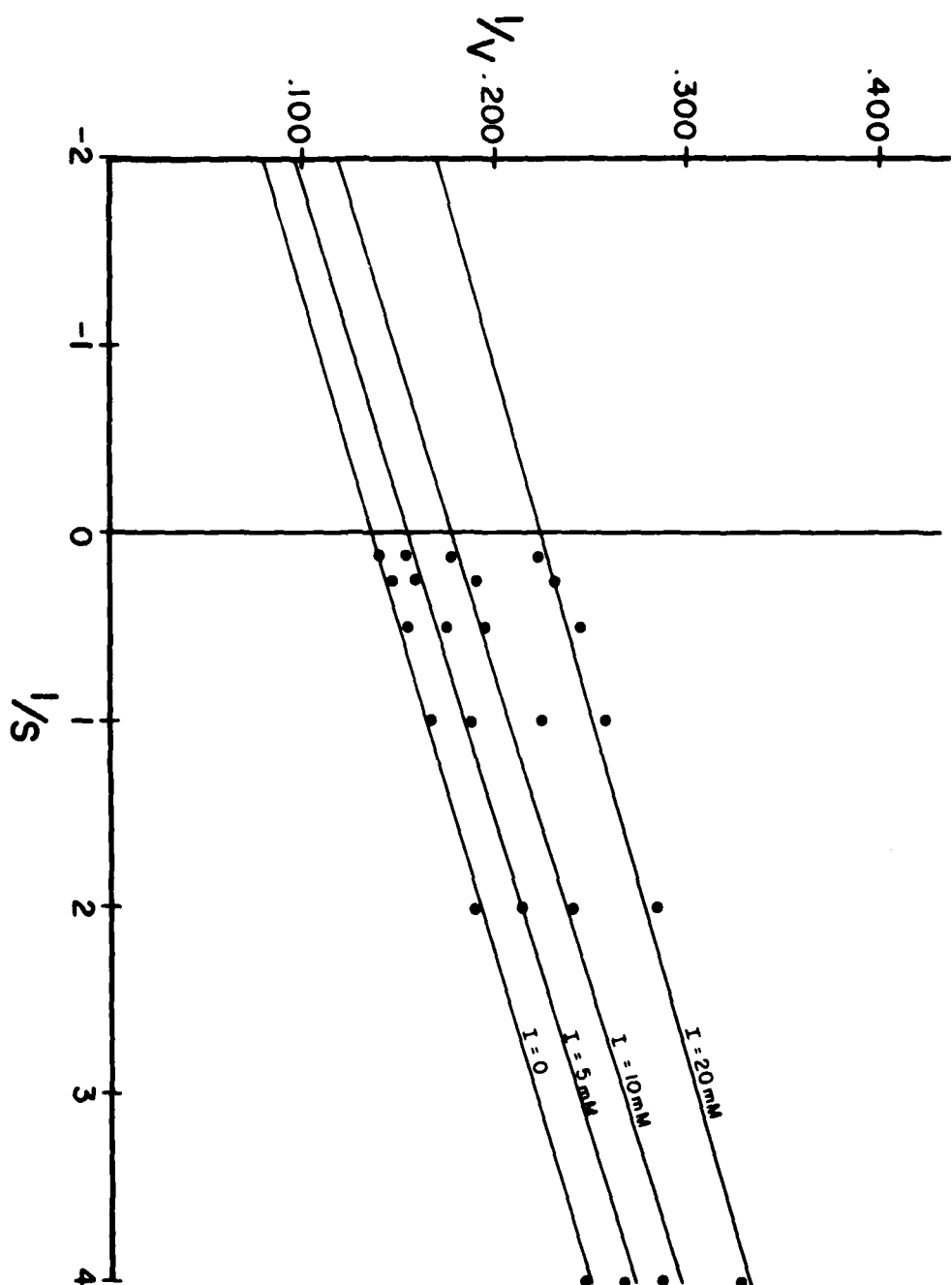


Fig. 2. Lineweaver-Burk plot ($1/\text{absorbance}$ vs $1/\text{millimoles} \cdot \text{liter}^{-1}$) of SQ-684 inhibition of human bone alkaline phosphatase.

Table 1. Sulfamylon Inhibition (%) of Alkaline Phosphatase from Different Tissues

Tissue	Sulfamylon Final Concentration			
	1 mM	5 mM	10 mM	20 mM
Liver	11.5 \pm 4.7	42.2 \pm 0.7	61.1 \pm 1.0	76.6 \pm 0.7
Granulation	12.0 \pm 0.8	43.8 \pm 1.2	63.1 \pm 0.9	76.6 \pm 0.9
Bone	10.6 \pm 4.8	43.5 \pm 0.7	60.2 \pm 2.2	75.3 \pm 0.9
Kidney	13.5 \pm 2.3	40.8 \pm 1.1	61.3 \pm 0.7	75.9 \pm 0.7
Intestine	11.5 \pm 2.2	37.2 \pm 0.5	56.1 \pm 0.4	69.8 \pm 1.3
Placenta	3.6 \pm 2.7	13.3 \pm 3.7	23.1 \pm 3.4	35.7 \pm 2.9

Granulation = Burn wound granulation tissue; Data represent mean \pm S.D., n = 3. % Inhibition = $\frac{\text{Activity (uninhibited)} - \text{Activity (inhibited)}}{\text{Activity (uninhibited)}} \times 100$

The metabolite of sulfamylon, p-carboxybenzene sulfonamide (16) is also an effective inhibitor (Table 2). Addition of alkyl groups to the sulfonamide nitrogen reduces the potency of inhibition of the (alpha-amino) toluene-sulfonamides. Under alkaline conditions this nitrogen has an unbonded electron pair available to chelate the zinc atom in the active site; steric hindrance may account for the progressively diminished effect of the (N-methyl) and (N-isopropyl) derivatives.

Table 2. Inhibition of Alkaline Phosphatase by Several Sulfonamides, Percent

Drug	Final Concentration in Assay			
	1 mM	5 mM	10 mM	20 mM
Sulfamylon	10.6 \pm 4.8	43.5 \pm 0.7	60.2 \pm 2.2	75.3 \pm 0.9
p- (N-methyl) ATSA	3.6 \pm 1.3	15.0 \pm 4.1	29.9 \pm 2.6	48.2 \pm 3.3
p- (N-isopropyl) ATSA	0.5 \pm 0.4	5.6 \pm 1.1	11.5 \pm 1.0	19.6 \pm 2.0
Furosemide	13.6 \pm 3.8	33.7 \pm 0.8	60.5 \pm 2.4	91.4 \pm 0.1
Acetazolamide	7.0 \pm 2.4	31.9 \pm 4.4	48.1 \pm 2.2	63.3 \pm 1.3
p-carboxybenzene SA	0.8 \pm 5.9	19.0 \pm 2.8	35.3 \pm 2.4	55.2 \pm 0.9
Sulfanilamide	4.8 \pm 2.0	21.2 \pm 4.0	34.3 \pm 3.2	49.8 \pm 3.7

ATSA = (alpha-amino)toluenesulfonamide; SA = Sulfonic acid; Data represent mean \pm S.D., n = 3

16. Hartles RL, Williams RT: Metabolism of sulphonamides: Relation of metabolic fate of ambamide (Marfanil) and V 335 to their lack of systemic antibacterial activity. *Biochem J* 41: 206-212, 1947.

Progressive dilution of sulfamylon-inhibited and non-inhibited AP indicate that the inhibition is reversible. At a dilution of 1:16, the inhibitor concentration was 0.5 mM and activity of the inhibitor-containing enzyme solution was the same as the control. To rule out inhibition by the counter ion, sodium acetate at final concentrations ranging from 1-20 mM was added to bone AP and found to have no inhibitory effect. Magnesium, which stimulates AP activity, did not, at concentrations ranging from 5×10^{-4} to 5×10^{-2} molar, affect the inhibition of bone AP by 10 mM sulfamylon. The acetazolamide inhibition of parathyroid hormone induced bone resorption has been interpreted in the context of the well known inhibition of carbonic anhydrase by sulfonamides (17). Minkin and Jennings recognized that their observations could be the result of another system which is part of the mechanism of bone resorption and which is also inhibited by sulfonamides. Considering the relationship between AP and inorganic phosphate, and reports which implicate AP as having some key role in calcium metabolism (5,18,19), the results of this study raise the possibility that the inhibition of bone resorption is due to inhibition of AP (rather than, or in addition to, inhibition of carbonic anhydrase). Further, furosemide blocks calcium reabsorption in the loop of Henle, leading to hypocalcemia and calciuria (20); it is possible that these effects are due to the inhibition of kidney AP rather than, or in addition to inhibition of carbonic anhydrase by furosemide.

PRESENTATIONS AND/OR PUBLICATIONS

None

17. Minkin C, Jennings JM: Cellular control of calcium movements in bone. *Science* 176: 1031-1033, 1972.

18. Ramp WK: Parathyroid hormone inhibition of induction of alkaline phosphatase activity by hydrocortisone and 5-iodo-2'-deoxyuridine in HeLa cells. *Clin Orthop* 106: 311-322, 1975.

5. Price GH: Studies on the mechanism of induction of alkaline phosphatase in HeLa cells. Dissertation for Ph.D., University of Oklahoma, 1972.

19. Streifler C, Orenstein A, Harell A: Carbonic anhydrase and bone remodeling: Sulfonamide inhibition of bone resorption in organ culture. *Acta Endocrinol* 70: 676-682, 1972.

20. Ong SC, Shalhoub RJ, Gallagher P, Antoniou D, O'Connell JMB: Effect of furosemide on experimental hypercalcemia in dogs. *Proc Soc Exp Biol Med* 145: 227-233, 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACRONYM		2. DATE OF SUMMARY		3. REPORT CONTROL NUMBER	
4. DATE PREVIOUS SUMMARY				5. AGENCY ACRONYM		6. DATE OF SUMMARY		7. REPORT CONTROL NUMBER	
77 10 01				DA OD 6978		78 10 01		DD DRAC (AR) 636	
8. KIND OF SUMMARY		9. SUMMARY SECTION		10. REPORT SECURITY		11. REGARDING		12. SPECIFIC DATA - CONTRACTOR ACCESS	
D. CHANGE		U		U		NA		NL	
13. NO. / CODES		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
6. PRIMARY		61102A		3S161102BS05		00		081	
7. CONTRIBUTING									
8. CONTRIBUTING									
14. TITLE (Provide with Security Classification Code) (U) Evaluation of Synthetic Sheeting as Operating Room Drape Material For Use in a Military Burn Unit (44)									
15. SCIENTIFIC AND TECHNOLOGICAL AREAS									
003500 Clinical Medicine									
16. START DATE			17. ESTIMATED COMPLETION DATE			18. FUNDING AGENCY		19. PERFORMANCE METHOD	
70 07			Cont			DA		C. In-House	
20. CONTRACT GRANT Not Applicable									
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C. KIND OF AWARD:				D. CUM. AMT.				E. FUNDING	
FISCAL YEAR				CURRENT YEAR				F. FUNDING	
78				79				8	
.3				.2				8	
24. RESPONSIBLE DOD ORGANIZATION					25. PERFORMING ORGANIZATION				
NAME: US Army Institute of Surgical Research					NAME: US Army Institute of Surgical Research				
ADDRESS: Ft Sam Houston, Texas 78234					ADDRESS: Ft Sam Houston, Texas 78234				
RESPONSIBLE INDIVIDUAL					PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)				
NAME: Basil A. Pruitt, Jr, COL, MC					NAME: Basil A. Pruitt, Jr, COL, MC				
TELEPHONE: 512-221-2720					TELEPHONE: 512-221-2720				
26. GENERAL USE					27. SOCIAL SECURITY ACCOUNT NUMBER:				
FOREIGN INTELLIGENCE NOT CONSIDERED					ASSOCIATE INVESTIGATORS				
					NAME: Robert B. Lindberg, PhD				
					NAME:				
28. KEYWORDS (Provide EACH with Security Classification Code) (U) Military burn unit; (U) Operating room based infections; (U) Surgical drapes; (U) Surgical gowns									
29. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number, precede text of each with Security Classification Code.)									
23. (U) Evaluation in terms of draping characteristics, absorbency, physician acceptance, and bacterial barrier qualities of a Spunbonded Olefin-cellulosic Laminated sheeting as surgical drapes and gowns. A decrease in bacterial seeding of operative wounds via drapes will minimize postoperative wound infections decreasing subsequent morbidity and mortality in injured troops.									
24. (U) Laboratory assessment of bacterial barrier properties of synthetic sheeting. Clinical use of drapes on burn patients to determine surgeon acceptability. Photographic documentation of draping characteristics, absorbency, and "run-off." Pre- and postoperative cultures at margin of operative field. Temperature monitoring to determine heat transmission characteristics.									
25. (U) 7710 - 7809 The bacterial barrier property of synthetic drape material has been further assessed using the testing regimen developed in this laboratory. This effect of agar concentration on drape penetrating by Pseudomonas sp. was studied using five agar concentrations ranging from 1.5% to 2.5%. Bacterial penetration appears to be minimally affected by agar concentration but significantly dependent on drape composition with an as yet undefined interaction apparent between agar concentration and one of the drape materials.									

* Available to contractors upon author's approval.

DD FORM 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 69 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: EVALUATION OF SYNTHETIC SHEETING AS OPERATING ROOM
DRAPE MATERIAL FOR USE IN A MILITARY BURN UNIT

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

Basil A. Pruitt, Jr., MD, FACS, Colonel, MC
Robert B. Lindberg, PhD
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05, MILITARY BURN RESEARCH

REPORT TITLE: EVALUATION OF SYNTHETIC SHEETING AS OPERATING ROOM DRAPE MATERIAL FOR USE IN A MILITARY BURN UNIT

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

Investigators: Basil A. Pruitt, Jr., MD, FACS, Colonel, MC
Robert B. Lindberg, PhD
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

The effect of culture media agar concentration on *Pseudomonas* penetrability of three coded samples of synthetic surgical drape material was assessed by a testing technique developed at this Institute. The transmission of *Pseudomonas* organisms suspended in liquid culture media and inoculated at six sites on circular samples of each of three drape materials was determined in four to six replicate trials of each material.

Penetration rates differed significantly between drape materials. One of the three materials possessed clearly superior barrier properties with penetration identified at only 10 per cent of all inoculation sites. The other two materials possessed minimal barrier properties with transmission through 151 of 180 (84 per cent) inoculation sites and 119 of 120 (99 per cent) inoculation sites, respectively. The effect of five concentrations of agar ranging from 1.5 to 2.5 per cent on bacterial transmission was evaluated. Agar concentration appeared to influence *Pseudomonas* penetrability only in the tests of one of the drape materials. Drape composition (density) appeared to influence *Pseudomonas* penetrability to a far greater extent than agar concentration. Further testing of the more effective barrier material is anticipated.

Military burn unit
Operating room based infections
Surgical drapes
Surgical gowns

EVALUATION OF SYNTHETIC SHEETING AS OPERATING ROOM DRAPE MATERIAL FOR USE IN A MILITARY BURN UNIT

Standard surgical drapes, when they become moistened in the course of operation, readily permit passage of microorganisms and are inadequate protective barriers of surgical wounds. Synthetic drape materials possessing superior barrier properties have been increasingly accepted and are now used in approximately 50 per cent of all operations. However, the processes required to soften the synthetic materials for improved draping characteristics and to bond cellulosic materials to the synthetics for improved absorbency to limit fluid run-off have compromised the barrier properties of such materials. Testing of newer forms of spun-bonded Olefin has been carried out to evaluate the characteristics important in drape material barrier reliability. Differences in test results suggested that the agar concentration of the culture media upon which the microbial penetration tests were performed might influence the results. The effect of agar concentration on bacterial penetrability of three drape materials has accordingly been evaluated.

Methods

Discs 85 mm in diameter were cut from each of three coded synthetic sheeting material samples and sterilized with ethylene oxide in glass Petri dishes. An assay strain of *Pseudomonas aeruginosa* was activated from frozen stock and passed serially through broth three times prior to use in testing. Discs of each drape material were placed individually on the surface of blood agar plates and drops of 0.07 ml of the broth culture were placed equidistant in a circle 1 cm from the edge of the material with six drops used on each plate. Five concentrations of agar were used in the culture media for the penetrability tests, 1.5 per cent, 1.75 per cent, 2 per cent, 2.25 per cent and 2.5 per cent. Four to six replications of the test were made for each concentration of agar, using each of the test materials.

Following four hours exposure of the material to the broth culture at room temperature, any remaining fluid was removed with a Pasteur pipette and the disc of drape material removed from the agar plate with care taken to prevent inadvertent contamination of the agar. The agar plates were then incubated at 37°C for 24 hours and growth of bacteria identified when present at each individual inoculation site. If confluent growth occurred extending to two inoculation sites, each of the sites involved was rated as positive, although it was theoretically possible for a single breakthrough to spread over adjacent sites under the paper disc.

Results

The percentage of penetration of inoculation sites of each sheeting sample and for each concentration of agar is shown in the Table. As can be seen, sample 977B was the most effective barrier with penetration of only 10 per cent of all inoculation sites. The other two drape materials possessed minimal and essentially no barrier function, respectively, with 84 per cent penetration of all sites for drape material 977E and 99 per cent penetration for drape material 977I. Bacterial penetration appeared to be only slightly effected by agar concentration with a penetration of 66 per cent in those tests using 1.5 per cent agar, decreasing to 52 per cent in those tests in which 2.5 per cent agar was used. This difference appeared to be related largely to results of tests in which drape material 977E was used.

TABLE I
EFFECT OF AGAR CONCENTRATION AND DRAPE MATERIAL
ON PSEUDOMONAS PENETRABILITY

Agar Concentration	Drape Material			Total	% Penetration
	977B*	977E*	977I*		
1.5%	4/36	35/36	24/24	63/96	66
1.75%	2/36	36/36	24/24	62/96	65
2.00%	4/36	33/36	24/24	61/96	64
2.25%	4/36	24/36	24/24	52/96	54
2.5%	4/36	23/36	23/24	50/96	52
TOTAL	18/180	151/180	119/120		
% PENETRATION	10	84	99		

* Sites of Penetration/Total sites inoculated

Discussion

Pseudomonas aeruginosa organisms were chosen for this assessment of bacterial penetration of drape material as related to agar concentration because that organism, on the basis of previous testing, has been found to be the most adept of several gram-negative organisms in traversing drape material. The three drape materials showed considerable variation

in *Pseudomonas* penetrability which ranged from only 10 per cent with material 977B to 99 per cent (essentially no barrier) with material 977I. These findings confirm the importance of drape material composition and characteristics in barrier property function of surgical drapes.

A difference in penetrability testing results between those obtained at this Institute and at another laboratory using different culture media, prompted assessment of the effect of agar concentration on drape material penetration by *Pseudomonas* sp. The data from these tests suggests that there may be an interaction between drape material and agar concentration. A variation in penetrability as related to agar concentration was evident only in tests of material 977E. Even in those tests the observed differences in penetrability as related to agar concentration were small relative to those differences related to drape material.

In summary, these findings confirm our earlier studies of various drape materials indicating that drape fabrication and density are of critical importance in terms of bacterial barrier properties. The samples of material 977B on gross assessment appeared to be of greater density than either of the two other materials. The effect of agar concentration in the test media was evident only in tests of material 977E and appeared to be of relatively minor importance compared to the variations associated with drape material. Further testing is anticipated of the more effective barrier material during the forthcoming year.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				DA OG 6972	78 10 01	REPORT CONTROL SYMBOL FDI-PAE(AR)636	
1. DATE PREP. SUMMARY 77 10 01	2. KIND OF SUMMARY D. CHANGE	3. SUMMARY CLASS U	4. SECURITY CLASS U	5. REPORTING NA	6. LIMITATION NL	7. SPECIFIC DATA X-TRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	8. LEVEL OF SUMMARY A. WORK UNIT
10. NO. / CODES A. PRIMARY B. CONTRIBUTING C. CONTRIBUTING	PROGRAM ELEMENT 62774A	PROJECT NUMBER 3S162774A820	TASK AREA NUMBER 00	WORK UNIT NUMBER 116			
11. TITLE (Precede with Security Classification Code) (U) Studies of Infection and Microbiologic Surveillance of Troops With Thermal Injury (44)							
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13. START DATE 76 10		14. ESTIMATED COMPLETION DATE Cont		15. FUNDING AGENCY DA		16. PERFORMANCE METHOD C. In-House	
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Not Applicable				PRECEDING			
EXPIRATION:				FISCAL YEAR			
A. AMOUNT:				78 6.8 225			
F. CUM. AMT.				79 4.3 156.4			
19. RESPONSIBLE DCO ORGANIZATION NAME: US Army Institute of Surgical Research ADDRESS: Ft Sam Houston, Texas 78234 RESPONSIBLE INDIVIDUAL NAME: Basil A. Pruitt, Jr., COL, MC TELEPHONE: 512-221-2720				20. PERFORMING ORGANIZATION NAME: US Army Institute of Surgical Research Microbiology Branch ADDRESS: Ft Sam Houston, Texas 78234 PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution) NAME: Robert B. Lindberg, PhD TELEPHONE: 512-221-2018 SOCIAL SECURITY ACCOUNT NUMBER: ASSOCIATE INVESTIGATORS NAME: NAME:			
21. GENERAL USE FOREIGN INTELLIGENCE NOT CONSIDERED				DA			
22. KEY WORDS (Precede EACH with Security Classification Code) (U) Pseudomonas; (U) Klebsiella; (U) Staphylococci; (U) Wound Infection; (U) Sepsis; (U) Endotoxin; (U) Topical chemotherapy; (U) Humans							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.) 23. (U) Burns constitute a large component of military injuries in combat. The military relevance of this research lies in the fact that infection and resultant sepsis are major problems in burned soldiers. Control of surface infection has been pursued empirically. At the same time, mechanisms of infection, epidemiology, topical chemotherapy and anti-biotic use are assessed in relation to causative bacterial species. 24. (U) Culture of burn wound surfaces, sputum, blood, biopsies, urine and other sites related to infection are done. Detailed strain differentiation by multiple technics is done. Endotoxin is assayed. Virulence is assessed on experimental burn wound models, and new drugs are assessed on standardized infection models in burned animals. 25. (U) 7710 - 7809 Epidemic <u>Pseudomonas aeruginosa</u> episodes occurred in burn wounds despite intensive Silvadene (R) topical therapy. A mixed regimen in which Silvadene and Sulfamylon were alternated appeared to control this epidemic trend. Increase in oxidative and exotic fermentative species was found. Sulfamylon resistance did not occur, but silver resistance increased. <u>Staph. aureus</u> monotype epidemics were conspicuous. Methicillin resistance disappeared, but Cleocin resistance appeared abruptly. Fungi were found in 20% of tissues examined; candida sp. predominated. New metalsulfanamide compounds (Yu, Cu, Cr) were highly effective in control of experimental burn wound sepsis. Phage-typing of <u>Serratia marcescens</u> was brought to a definitive level, and sero-typing of <u>Ps. aeruginosa</u> was refined to a point where epidemic strains could be differentiated.							

* Available to contractors upon originator's approval

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ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- ANTIBIOTIC
SENSITIVITY OF CURRENT MILITARY BURN PATIENT FLORA

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

Robert B. Lindberg, PhD
Jack Henderson, PhD
Jimmie D. Cantrell, SP6
William S. Hardy, SP5
Mary D. Goff, SP4

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- ANTIBIOTIC SENSITIVITY
OF CURRENT MILITARY BURN PATIENT FLORA

It is a truism that bacterial infection is the most common cause of death in severely burned patients. Prompt recognition of presence of invading microorganisms, their identification and the delineation of their antibiotic sensitivity, are of paramount importance in controlling sepsis in the burn patient. This emphasis is strengthened by the consensus that use of prophylactic antibiotics in the uninvaded patient is counter-productive. Antibigrams in this Institute are determined primarily on blood stream isolates. Out of the total of 630 strains tested in 1977-78, 18 strains from 11 patients were from wounds rather than blood cultures. Antibigrams are a useful aid in strain differentiation in populations subject to cross-contamination. In the Institute of Surgical Research (ISR), both short- and long-term shifts in sensitivity have occurred. Sensitive populations have shifted to resistant, and the reverse pattern has also been seen. A long-term trend in the direction of increasing resistance, especially among gram-negative bacilli, has been evident in the past decade, but exceptions occur often enough to make a long-term prediction uncertain. Antibigrams have been of real aid in characterizing epidemic outbreaks in this Institute.

TECHNIC

A Minimum Inhibitory Concentration (MIC) has been described (1). Antibiotic test solutions are made up in Trypticase Soy Broth (TSB) and dispersed in 10 mm X 60 mm tubes in 1 ml amounts, then quick-frozen and stored at -20°C , in concentrations diluted from 50 ug/ml to 0.78 ug/ml. In use, a dilution series is thawed at room temperature and seeded with 1 ml of an 18 hour broth culture at a concentration of 10^5 organisms/ml. Inhibitory end points are read after 18-20 hours incubation.

Seventeen species of bacteria, and a total of 630 strains were tested. Results, shown in Table 1, reflect the fact that only four species of bacteria appeared with sufficient frequency to constitute a meaningful occurrence. There were 128 patients with positive blood cultures, or 46.3% of the 276 patients admitted during this period. Staphylococcus aureus was the overwhelmingly predominant species; it involved twice as many patients as had been positive in recent years. Staphylococcus epidermidis was numerically important, although it was still equivocal as a pathogen. Nevertheless, it was the second most frequent organism in terms of patients involved. Pseudomonas aeruginosa

1. Lindberg RB, Contreras AA, Smith HOD, Jr, Plowey EC, Mason AD, Jr: Antibiotic sensitivity of current military burn patient flora. USA Inst Surg Res Annual Rpt FY 1973. Brooke Army Medical Center, Fort Sam Houston, Texas. Section 7.

ABSTRACT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- ANTIBIOTIC
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Six hundred and thirty strains of bacteria, recovered primarily from blood cultures, were assessed for antibiotic sensitivity by the Minimum Inhibitory Concentration (MIC) technic. Staphylococcus aureus and Pseudomonas aeruginosa were the principal species involved in sepsis. In contrast to previous years, Enterobacteriaceae spp. were not present in epidemic patterns. S. aureus were resistant to the aminoglycoside antibiotics. Methicillin group resistance was manifest as total resistance to nafcillin and extensive oxacillin resistance, even though there was relative sensitivity to methicillin. Minocin was entirely effective against staphylococci, but vibramycin, also tetracycline, was effective in less than half of the strains. Vancomycin remained entirely effective. Keflin remained highly effective, while clindamycin resistance appeared and became complete. Pseudomonas strains were aminoglycoside resistant. Minocin and vibramycin were active, as was colistin. Carbenicillin inhibited 62% of all strains. Klebsiella and Enterobacter were most inhibited by tetracycline. Therapeutic response in gram-negative sepsis, even when a strain was sensitive in vitro, was not encouraging.

Antibiotic sensitivity
Pseudomonas
Burn wounds
Chemotherapy
Staphylococci

Table 1. Species of Bacteria Tested for Antibiotic MIC,
1 October 1977 - 30 September 1978: Number of Patients
Positive and Number of Strains Recovered

Species	Year, No. of Patients, and No. of Strains ()			
	1975	1976	1977	1978
<i>S. aureus</i>	43 (153)	43 (125)	50 (75)	86 (345)
<i>S. epidermidis</i>	6 (6)	21 (36)	28 (66)	39 (52)
<i>Strep. spp. (a)</i>	7 (13)	9 (13)	15 (31)	19 (31)
<i>Corynebacterium sp.</i>	0	1 (1)	0	4 (4)
<i>P. aeruginosa</i>	45 (95)	42 (94)	42 (90)	31 (71)
<i>E. coli</i>	8 (9)	10 (11)	15 (23)	19 (45)
<i>Proteus sp. (b)</i>	4 (7)	6 (11)	10 (19)	12 (20)
<i>K. pneumoniae</i>	58 (159)	60 (145)	19 (32)	14 (25)
<i>Enterocloacae</i>	31 (62)	2 (2)	28 (41)	9 (19)
<i>Serratia sp.</i>	7 (22)	2 (4)	7 (10)	2 (4)
<i>Citrobacter sp.</i>	0	1 (2)	8 (15)	2 (3)

(a) *Strep. spp.* included Gp D Enterococcus (7 strains); Gp D (3); Gp B (2) and *S. pneumoniae* (4).

(b) *Proteus mirabilis*, 15 strains; *P. morgani*, 3; *P. rettgeri*, 2.

NOTE: Infrequent blood stream isolates, 1978: *P. cepacia*, 4; *P. maltophilia*, 1; *Acinetobacter sp.*, 1; *Aeromonas sp.*, 2; Gp VE-11, 1. 18 strains from 11 patients were from wounds, not blood cultures.

aeruginosa, which had been recovered at a relatively constant rate since 1975, decreased in incidence in blood cultures in 1978. *Enterobacteriaceae spp.* had caused several major epidemic episodes since 1970, but in the current reporting period, their role diminished. *Escherichia coli* recovered from 19 patients, was the most common species. *Klebsiella pneumoniae* and *Enterobacter cloacae*, each of which had in succession caused a long-term epidemic in the burn ward since 1970, occasioned only brief outbreaks of cross-contamination as evinced by clusters of blood cultures positive at intervals. *Proteus mirabilis* was recovered at a frequency comparable to other enteric species. *Proteus rettgeri* and *Proteus morgani* appeared but were rare. Other species of gram-negative species represented episodes of minor importance.

SENSITIVITY OF BURN PATIENT FLORA TO ANTIBIOTICS

The sensitivity of the burn flora tested during 1977-1978 is summarized in the following tables. The level of sensitivity is shown in cumulative ascending increments. The definition of "sensitive" and "resistant" has been somewhat empirically designated for gram-positive organisms as 6.25 ug/ml of antibiotic, and for gram-negative bacilli, 12.5 ug/ml. The value obtained serves as a guide for selection of antibiotic if treatment is indicated, but it

cannot be too strongly emphasized that this is a clinical decision, not one made by the laboratory.

The set of antibiotics used in routine testing is subject to modification as dictated by changing susceptibility patterns and the availability of new antibiotics. In contrast to previous years, no changes in the test battery were made during 1977-1978. Table 2 presents the antibiotics used in the test battery.

Table 2. Antibiotics Used in MIC Assessment for Sensitivity:
1977-1978

<u>Gram-Positive Organisms</u>			<u>Gram-Negative Organisms</u>		
Gentamicin	:	G	Gentamicin	:	G
Methicillin	:	Sc	-		
Oxacillin	:	Ps	-		
Nafcillin	:	U	-		
Minocin	:	M	Minocin	:	M
Keflin	:	Kf	Keflin	:	Kf
Vancomycin	:	Va	-		
Vibramycin	:	Vb	Vibramycin	:	Vb
Tobramycin	:	To	Tobramycin	:	To
-			Kanamycin	:	K
-			Amikacin	:	Ak
-			Colistin	:	Co

P. aeruginosa : add Carbenicillin : Cb
Proteus mirabilis: add Penicillin G : Pen G

Staphylococcus aureus: The continued succession of changes in sensitivity of staphylococci, especially with the methicillin group of semi-synthetic penicillins, has made this species of particular interest. The evident preponderance of staphylococci in bacteremia in this reporting interval makes detailed study of these strains of particular importance. Table 3 presents the sensitivity of 345 strains tested. Minocin, Keflin and vancomycin were the antibiotics unequivocally active in this population. Oxacillin was effective with three-fourth of the strains, and vibramycin with almost half. Only a third of the strains were sensitive to methicillin, and resistance to nafcillin was virtually total. Thus, these three methicillin-group antibiotics spanned the gamut of effectiveness, from 77% for oxacillin to 0.5% for nafcillin. This range of sensitivity places in question the widely held view that sensitivity to the methicillin group of antibiotics can be assessed by using only one antibiotic. The strains were virtually all resistant to gentamicin. The other aminoglycoside, tobramycin, similarly inhibited only a small part of the staphylococci tested. Clindamycin, which in previous years had been highly

Table 3. *Staphylococcus aureus*: Cumulative Inhibitory Levels for
345 Strains: 1 October 1977 - 30 September 1978

Antibiotic Level ug/ml	Antibiotic and % Inhibited									
	G	M	Ps	Sc	U	Kf	Va	Vb	To	Cl
> 25	100	100	100	100	100	100	100	100	100	100
25	11.5	100	83.3	87.8	30.2	99.3	100	99	11.3	15.0
12.5	10.9	98.5	81.8	70.1	4.6	98.4	99.6	99.4	11.3	14.7
6.25	7.4	95.3	77.9	31.0	0.5	96.9	99.6	42.2	16.7	14.4
3.12	5.9	58.1	60.7	11.8	0	95.7	94.2	35.2	7.2	14.1
1.5	5.3	31.0	23.2	7.3	0	85.2	45.7	11.3	5.9	14.1
< 0.78	1.7	13.6	8.0	4.1	0	55.5	11.2	8.0	2.9	13.5
Total Tested	337	345	336	338	344	333	330	335	317	332

G: Gentamicin; M: Minocin; Ps: Oxacillin; Sc: Methicillin; U: Nafcillin; Kf: Keflin;
Va: Vancomycin; Vb: Vibramycin; To: Tobramycin; Cl: Clindamycin.

effective, was also effective against only 14% of the strains.

A retrospective comparison of sensitivities over several years can reveal epidemic trends and patterns of change in resistance which are not otherwise apparent. Such a comparison, essentially of septicemic strains of staphylococci, is presented in Table 4. The percentage of strains inhibited at 6.25 ug/ml is shown. Striking shifts in sensitivity of the S. aureus population have indeed occurred.

Table 4. Comparison of Sensitivity of Staphylococcus aureus to Antibiotics: 1971-1978

Antibiotic	Year and % of Strains Inhibited by 6.25 ug/ml							
	1971	1972	1973	1974	1975	1976	1977	1978
Gentamicin	50.0	35.0	67.9	92.2	38.8	50.0	30.6	7.4
Minocin	-	-	34.1	96.0	46.5	92.8	93.9	95.3
Methicillin	15.5	13.1	50.0	65.2	21.8	23.5	35.7	77.9
Oxacillin	20.1	18.8	69.7	82.6	73.6	70.5	65.1	31.0
Nafcillin	33.0	26.0	62.3	83.3	85.6	49.5	1.8	0.5
Keflin	56.4	22.6	72.1	90.4	97.2	94.0	97.1	96.9
Vancomycin	-	-	-	-	100	100	100	99.6
Vibramycin	-	-	-	-	78.3	94.2	96.9	42.2
Tobramycin	-	-	-	-	88.0	100	65.4	16.7
Clindamycin	-	-	40.7	95.8	98.0	95.6	97.1	14.4

Gentamicin had risen in effectiveness during 1973-1974, but sensitivity to it diminished during 1975-1977. In 1978, the drop in proportion sensitive strains was unprecedentedly great. Staphylococci were virtually all resistant to gentamicin. Tobramycin, which was highly effective in 1975-1976, has shown a parallel drop in number of sensitive strains recovered. Methicillin has fluctuated in effectiveness; in 1978, the proportion of strains sensitive rose to 78% from a 1977 value of 35%. Oxacillin, however, decreased markedly in the number of strains sensitive, and nafcillin, the third semi-synthetic penicillin in the test battery, remained, as it had in 1977, virtually inactive against staphylococci. The species certainly did not show parallel degrees of sensitivity to these three methicillin analogues.

Minocin and keflin remained highly effective against staphylococci, as did vancomycin. Vibramycin was effective against only 42% of strains, in contrast to almost total sensitivity seen in 1976-1977. Clindamycin, which had been highly effective in the preceding four years, became entirely ineffective early in 1978.

Staphylococcus epidermidis: The role of recovery of this species, which is regarded as minimally pathogenic, was higher than had been observed prior to 1976. Whether clinical sepsis can be caused in burn wounds with this species alone remains debatable. However, 8 out of 39 patients with S. epi-

Table 5. Staphylococcus epidermidis: Cumulative Inhibitory Levels for
54 Strains - 1977-1978

Antibiotic Level ug/ml	G	M	Ps	Antibiotic and % Inhibited				Va	Vb	To	Cl
				Sc	U	Kf					
> 25	100	100	100	100	100	100		100	100	100	100
25	77.7	100	94.1	72.5	68.0	100		100	98.0	73.4	24.0
12.5	77.7	100	92.1	60.7	32.0	100		100	98.0	73.4	24.0
<hr/>											
6.2	77.7	96.1	88.2	49.0	18.0	98.0		98.0	89.8	67.3	24
3.1	77.7	92.3	80.4	31.3	2.0	96.0		98.0	81.6	67.3	24
1.5	75.9	90.3	54.9	19.6	0	96.0		74.5	65.3	65.3	20
≤ 0.78	68.5	78.8	35.3	15.6	0	82.3		29.4	51.0	59.2	18
No. Tested	54	52	51	51	50	51		51	49	49	50

G: Gentamicin; M: Minocin; Ps: Oxacillin; Sc: Methicillin; U: Nafcillin; Kf: Keflin;
Va: Vancomycin; Vb: Vibramycin; To: Tobramycin; Cl: Clindamycin

dermidis recovered from blood had more than one culture positive. This suggests that the species may be more invasive than has been assumed.

The strains were predominantly sensitive to gentamicin, and less so to tobramycin. Minocin, keflin, vancomycin and vibramycin were highly effective. Oxacillin, methicillin and nafcillin decreased in effectiveness in that order, from 88% for oxacillin to 18% for nafcillin. Sensitivity varied more than with S. aureus, and the heterogeneous patterns observed reflected the various sources contributing these strains.

Streptococci. There were 31 strains of streptococci recovered from 19 patients in blood culture. The group was heterogeneous, the following identifications were made.

	<u>Strains</u>
Strep., no Gp A, B, or D	10
Strep. Gp B	2
Strep. Gp D, Not Enterococcus	6
Strep. Gp D, Enterococcus	8
Strep. pneumoniae	5

The sensitivity of this group of organisms was relatively low. The values observed are summarized in Table 6. At the 6.2 ug/ml level, fewer than half were sensitive to gentamicin, nafcillin, tobramycin or clindamycin. Methicillin and oxacillin inhibited half the strains at this level, while minocin, keflin, vancomycin and vibramycin were the most active antibiotics. More precise differentiations of streptococcal species has resulted in recognition of 5 small sets of patients with different species recovered from the blood. Epidemic streptococcal sepsis has not been seen in burn patients under conditions prevailing in this Institute.

Pseudomonas aeruginosa. This organism has continued to occupy a uniquely conspicuous position in burn wound infections. During 1977-1978, it was the most numerous gram-negative species causing bacteremia, although this numerical importance diminished from the level seen in the previous 3 years. Thirty-one patients yielded a total of 71 strains in blood culture. Pseudomonas has long been noted for its high level of antibiotic resistance. However, in 1977-1978, out of 9 antibiotics used with Pseudomonas, 5 were inhibitory to a significant degree: Minocin, amikacin, vibramycin, colistin and carbenicillin (Table 7). Only one of the 4 aminoglycosides, amikacin, used was effective. Both of the tetracyclines, minocin and vibramycin, were at the upper level of effectiveness against Pseudomonas, and merit careful consideration for use in treatment of sepsis due to this opportunistic pathogen. Colistin was the most effective antibiotic tested, but this antibiotic has found little application despite its high in vitro activity. Carbenicillin was effective at the 156 ug/ml level with 62% of the strains. This valuable antibiotic has fluctuated in effectiveness over several years, but no progressive increase in resistance has occurred during the past 6 years.

Table 6. Streptococci, Heterogeneous: Cumulative Inhibitory Levels
for 31 Bacteremic Strains

Antibiotic Level ug/ml	G	M	Ps	Antibiotic and % Inhibited				Va	Vb	To	Cl
				Sc	U	Kf					
> 25	100	100	100	100	100	100		100	100	100	100
25	59.3	100	75.8	61.2	48.2	96.7		100	100	42.4	36.6
12.5	56.2	96.8	65.5	58.0	36.4	87.0		96.8	93.1	39.3	36.6
<hr/>											
6.2	43.7	86.6	51.7	54.8	29.0	64.5		96.6	82.7	36.3	36.6
3.1	37.5	76.6	48.2	54.8	25.8	64.5		93.3	82.7	83.3	33.3
1.5	28.1	66.6	44.8	48.3	25.8	58.0		66.6	73.3	27.2	30.0
< 0.78	21.8	63.3	31.0	32.2	25.8	54.8		56.6	62.0	24.2	20.0

G: Gentamicin; M: Minocin; Ps: Oxacillin; Sc: Methicillin; U: Nafcillin; Kf: Keflin
Va: Vancomycin; Vb: Vibramycin; To: Tobramycin; Cl: Clindamycin

Table 7. *Pseudomonas aeruginosa*: Cumulative Inhibitory Levels for 71 Strains from Blood Cultures, 1977-1978

Conc. ug/ml	C	K	Antibiotic and % Inhibited				Ak	Vb	Co	Conc. ug/ml	Cb	
			Kf	M	To							
> 25	100	100	100	100	100	100	100	100	>1250	100		
25	21.1	17.9	0	95.4	20.2	88.5	87.8	91.3	1250	98		
<hr/>												
12.5	19.7	5.9	0	77.2	17.0	60.0	63.6	91.3	625	92		
6.2	11.2	1.4	0	31.8	11.5	40.0	13.6	89.8	312	64		
3.1	5.6	0	0	4.5	8.6	7.1	1.5	89.9	156	62		
1.5	4.2	0	0	0	8.6	1.4	0	78.2	78	60		
< 0.78	0	0	0	0	2.8	0	0	53.0	39	58		
											19	22
											9	2.0
											49	0
<hr/>												
Total Tested	71	67	65	66	69	70	66	69			50	

G: Gentamicin; K: Kanamycin; Kf: Keftin; M: Minocin; To: Tobramycin;
 Ak: Amikacin; Vb: Vibramycin; Co: Colistin; Cb: Carbenicillin

Since antibiotic resistance has been of especial concern with P. aeruginosa, a comparison of sensitivity levels over the past 6 years provides a more significant view than can be seen in one annual increment. Table 8 presents the antibiotic sensitivity for aminoglycosides, tetracyclines and colistin for the past 5 years.

Table 8. Sensitivity of Pseudomonas aeruginosa to 3 Aminoglycosides, 2 Tetracyclines, Colistin and Carbenicillin

Antibiotic	Year and % of Strains Inhibited by 12.5 ug/ml				
	1973	1974	1975	1976-77	1978
Gentamicin	84.3	61.8	40.0	19.1	19.7
Tobramycin	-	-	18.5	61.6	17.0
Amikacin	-	-	-	98.3	60.0
Minocin	31.3	15.7	16.8	58.9	72.2
Vibramycin	-	-	20.0	43.6	63.6
Colistin	86.2	93.3	86.3	89.3	91.3
Carbenicillin	80.4	70.8	68.8	58.6	62.0

Sensitivity cutoff: 156 ug/ml

The most striking change observed in this period was the loss of effectiveness of gentamicin. From a high point of 84.3% of strains sensitive in 1973, it fell to 19.1% in 1976. Tobramycin and amikacin were more recently added to the aminoglycoside battery. Tobramycin has fluctuated in effectiveness, but its activity has been minimal in 1978. Amikacin was completely effective when first used; its activity fell to 60% of strains in 1978. The tetracycline, minocin, was initially relatively inactive, but sensitivity has increased strikingly in the past 2 years. This pattern was also seen with vibramycin, a tetracycline. Colistin has long been one of the most effective in vitro antibiotics for Pseudomonas. However, it is seldom used in therapy. Carbenicillin was at peak effectiveness in 1973, but since then only 60% to 70% of strains have been sensitive.

Klebsiella pneumoniae. This species diminished markedly in incidence as a cause of sepsis in burns in 1977-78. It is still an ominous harbinger of a fatal outcome; most patients with Klebsiella bacteremia do not survive. But only 25 strains were recovered from blood cultures from 14 patients in 1977-78; in contrast to the 20 month preceding interval in which 62 patients yielded 175 strains (Table 9). There was a striking change in sensitivity to gentamicin. In 1976-1977, only 7% of

Table 9. *Klebsiella pneumoniae*: Cumulative Inhibitory Levels for
25 Strains from Blood Cultures. 1977-1978 (Oct-Sep)

Conc. ug/ml	G	M	K	Antibiotic and % Inhibition			Ak	Co
				Kf	To	Vb		
> 25	100	100	100	100	100	100	100	100
25	66.6	100	60.8	62.5	70.8	82.6	88.0	80.0
12.5	58.3	83.3	56.5	58.3	58.3	78.2	88.0	80.0
6.2	54.1	70.8	52.1	54.1	50.0	78.2	72.0	80.0
3.1	45.8	45.8	43.4	29.1	41.6	47.8	40.0	76.0
1.5	37.5	25.0	21.7	0	33.3	34.7	12.0	68.0
< 0.78	8.3	4.1	0	0	12.5	13.8	0	52.0
No. Tested	24	24	23	24	24	23	25	25

G: Gentamicin; M: Minocin; K: Kanamycin; Kf: Keflin; To: Tobramycin;
Ak: Amikacin; Co: Colistin

strains were sensitive; in the 1977-78 period, 58% of the strains were sensitive to 12.5 ug/ml. Of the other aminoglycosides, kanamycin shifted from 2% to 56% sensitive. Tobramycin increased from 37% to 58% effective. Amikacin continued to be highly effective against *Klebsiella*, as did minocin and vibramycin, representing tetracyclines. Keflin had been virtually ineffective; sensitivity to this agent increased from 9% of strains in 1976-77 to 58% in 1977-78. The change to a more susceptible population occurred precisely at the time when epidemic *Klebsiella* infection ceased in this population. The more sensitive strains may well represent a heterogeneous population with only an occasional strain causing sepsis. As with other species, this return to sensitivity of a previously resistant population was not associated with a discernible change in clinical use of antibiotics.

Enterobacter cloacae. As was the case with *K. pneumoniae*, this enteric species had established major epidemics on the burn ward but in the 1977-1978 period, its appearance was sporadic. Nine patients yielded 19 septicemic strains. These isolates were relatively resistant to antibiotics; 15% were sensitive to gentamicin and to kantrex, and 21% to tobramycin. Only amikacin among the aminoglycosides was active; 89% of the strains were inhibited. Minocin, with 95% of strains sensitive, and vibramycin with 89%, indicated that tetracyclines merited consideration as antibiotics when *Enterobacter* sepsis is encountered. Colistin also inhibited 89% of the strains.

Escherichia coli. Since 1976, *E. coli* bacteremia has been observed with a frequency comparable to the current incidence of *Klebsiella* or *Enterobacter* sepsis. In 1977-78, 17 patients yielded 45 strains from blood culture.

Table 10 summarizes the sensitivity data for *E. coli*. Gentamicin, out of the aminoglycoside antibiotics used, was by far the most effective. Kantrex, tobramycin and amikacin each inhibited approximately half of these septicemic strains. Minocin was also inhibitory to half of the strains, while vibramycin was effective with only 35% of strains tested. Keflin was more active than any other antibiotics except gentamicin and colistin. There was a significant increase in resistance on the part of strains of *E. coli*, over the sensitivity observed in 1976-1977. Only gentamicin was an exception to this trend.

Proteus mirabilis. The incidence of *Proteus* spp. septicemia was approximately that seen in the previous year. Twelve patients yielded 20 positive blood cultures. Ten patients had *P. mirabilis* in blood, 3 harbored *Proteus morganii* and 2, *Proteus rettgeri*. The discrepancy in totals represents those patients who had two species recovered in successive blood cultures. Fifteen strains of *P. mirabilis*, 3 of *P. morganii* and 2 of *P. rettgeri* were recovered. *P. morganii* and *P. rettgeri* were each more resistant than were the *P. mirabilis* strains. Table 11 summarizes the sensitivity patterns of *Proteus* spp. Gentamicin, keflin and vibramycin were the effective aminoglycosides. Amikacin was only inhibitory with 16% of strains. The tetracyclines, minocin and vibramycin, in the same high effective range as gentamicin. Colistin-resistance was complete.

Table 10. Escherichia coli: Cumulative Sensitivity of 45 Strains
from Blood Cultures of 17 Patients, 1977-1978

Conc. ug/ml	G	M	K	Antibiotic and % Inhibited				Ak	Co
				Kf	To	Vb			
>25	100	100	100	100	100	100	100	100	100
25	91.1	95.4	48.8	82.2	55.3	60.0	76.7	88.8	
12.5	88.8	52.2	46.6	64.4	53.3	35.5	48.8	88.8	
6.2	60.0	43.2	44.4	33.3	44.9	29.4	30.2	84.4	
3.1	31.1	31.8	17.7	6.6	17.7	24.4	4.6	84.4	
1.5	6.6	22.7	2.2	0	2.2	20.0	0	84.4	
<0.78	2.2	22.7	2.2	0	0	2.2	0	75.5	

G: Gentamicin; M: Minocin; K: Kanamycin; Kf: Keflin; To: Tobramycin;
Ak: Amikacin; Co: Colistin

Table 11. Proteus spp.: Cumulative Sensitivity of 20 Strains
Recovered in Blood Cultures. 1977-1978

Conc. ug/ml	G	M	K	Antibiotic and % Recovered			Ak	Co
				Kf	To	Vb		
>25	100	100	100	100	100	100	100	100
25	78.9	78.9	68.4	85.0	75.0	70.0	22.2	0
12.5	77.7	78.9	68.4	35.0	55.0	70.0	16.6	0
6.25	68.4	68.4	50.0	10.0	55.0	40.0	11.1	0
3.12	36.8	52.6	27.7	0	55.0	20.0	5.5	0
1.56	21.0	31.5	0	0	30.0	5.0	0	0
<0.78	0	5.2	0	0	5.0	0	0	0
No. Tested	19	19	18	20	20	20	18	19

G: Gentamicin; M: Minocin; K: Kanamycin; Kf: Kofing; To: Tobramycin

Ak: Amikacin; Co: Colistin

Infrequent Gram-negative Bacilli. There were two more species of Enterobacteriaceae from blood cultures: Serratia marcescens (3 patients, 4 strains) and Citrobacter diversus (2 patients, 3 strains). Two pseudomonads, Pseudomonas maltophilia (1 patient) and Pseudomonas cepacia (3 patients, 4 strains) were also recovered. One Aeromonas sp, one Acinetobacter sp. and one CDC VE-HII completed the collection of infrequent species. These samples were too small for significance in sensitivity testing. The pseudomonads were sensitive to all 8 antibiotics, as were Citrobacter and VE-HII. Aeromonas and Acinetobacter were resistant to all antibiotics except minocin and vibramycin.

DISCUSSION

During October 1977 - September 1978, S.aureus and P.aeruginosa were the major species involved in sepsis in burn patients. In previous years, species of Enterobacteriaceae had caused successive epidemics in the burn ward; this year, K.pneumoniae, Enterocloacae and Proteus spp appeared only in small sporadic episodes. No predominance of any one species occurred. E.coli was the most common enteric organism in burn patients, but did not appear in epidemic pattern. Antibiotic sensitivity in staphylococci showed the most effective agents to be the tetracyclines minocin and vibramycin, keflin, and vancomycin. The aminoglycosides gentamicin and tobramycin were ineffective. Methicillin resistance was marked from October to December, then in January sensitivity reappeared and lasted until April 1978. At that point resistance reappeared, to last for three months, after which the population was mixed sensitive and resistant. Oxacillin sensitivity paralleled this sequence, but nafcillin was ineffective for the whole year. Clindamycin exhibited a striking pattern: strains were highly sensitive up to October 1977, then two episodes of resistance were noted., after which total resistance appeared, and has since persisted.

P. aeruginosa was highly resistant to gentamicin, kantrex, keflin and tobramycin. Minocin exhibited a shift of striking intensity. The strains were resistant over a 6-month period. Sensitivity then reappeared and lasted for the rest of the year. Vibramycin sensitivity paralleled this pattern, but sensitivity was not as high as it was for minocin. Amikacin fluctuated, with resistance alternating with sensitivity at irregular intervals. By September 1978, sensitive strains had disappeared.

K. pneumoniae strains were most sensitive to vibramycin, amikacin, minocin and colistin. Approximately half of the strains were sensitive to gentamicin, kantrex, keflin and tobramycin. Enterocloacae was sensitive to amikacin, minocin and vibramycin in parallel with the pattern seen with Klebsiella.

E. coli, which became more frequent in wound infection in 1977-1978, was most often sensitive to gentamicin. Keflin inhibited 64% of strains at 12.5 ug/ml. Keflin and colistin were the only strikingly effective antibiotics with the remainder inhibiting only approximately half of the strains. Proteus spp., the other species recovered in significant numbers, was relatively

sensitive to gentamicin, but less so to tobramycin. Minocin and vibramycin were also effective.

Minor species of Enterobacteriaceae varied in antibiotic response. These forms were not sufficiently common to permit generalization regarding plausible antibiotics to be used.

PUBLICATIONS - None

PRESENTATIONS

Lindberg RB: Current status of antibiotics and burn infection. Symposium on Impact of Infections on Medical Care in the U.S. May 30-31, 1978, Washington, D.C.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- BACTERIOPHAGE TYPES
OF PSEUDOMONAS AERUGINOSA FOUND IN BURNED SOLDIERS

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ABSTRACT

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Period covered in this report: 1 October 1977- 30 September 1978

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The potential serotyping of *Pseudomonas aeruginosa*, using the 17-serum set proposed by the International Panel of the Subcommittee on *Pseudomonadaceae*, has been explored, with 477 strains from clinical specimens typed. The number of types that can be differentiated was enlarged by using multiple type formulae. Twenty-one types were differentiated. Major epidemic episodes were caused by strains of type 4 and 15; in addition, well-defined outbreaks due to type 6, 9-10, and 11 were distinguished. The technic discloses a pattern of epidemic spread similar to that revealed by phage typing; the similarity makes it more probable that the hypothesis of the occurrence of strain-specific microepidemics in burn wards is correct.

Pseudomonas
Serotype
Phage type
Infection
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF
TROOPS WITH THERMAL INJURY -- BACTERIOPHAGE TYPES OF
PSEUDOMONAS AERUGINOSA FOUND IN BURNED SOLDIERS

Pseudomonas aeruginosa as inciter of invasive infection and ultimate sepsis in severe burns, has remained important despite continued availability of antimicrobial agents which exhibit effective in vitro spectra of inhibition. Previous observations have established the existence of a pattern of intermittent acute epidemic outbreaks in the Institute of Surgical Research (ISR) burn wards, with intervening periods during which a heterogeneous population pervades the susceptible patients. The natural history of contagious epidemics is one of selective colonization by specific strains which are transferred from patient to patient to establish a predominant population which most often is transient, i. e., these episodes may take place in a period of one to several weeks. Phage type differentiation showed that epidemic strains seldom recurred, but serotype differentiation suggests that certain epidemic types may recur year after year.

The ongoing surveillance of Pseudomonas strains is dictated by the ubiquitous and recurrent colonization by these organisms on burn ward patients. Serotyping is a simpler tool than phage typing and has been applied to a representative sample of the burn wound flora recovered during the period August 1977 - July 1978.

METHODS

The typing sera used were obtained from Difco Laboratories, licensed manufacturers of the 17-serum typing set proposed by the International Panel of the Subcommittee on Pseudomonadaceae. These antisera are prepared from autoclaved antigens to avoid the incursion of thermolabile surface components of the cell wall. If both live and autoclaved test suspensions of bacteria being typed are used, a larger proportion of the population will be found to react. After 2 years of experience, it is our recommendation that typing be done with live suspensions; non-reactive strains can have the test suspension autoclaved, the cells washed and the suspension re-tested. This routine obtained the largest number of positive reactions with the least amount of manipulation.

RESULTS

Four hundred seventy-seven strains of P. aeruginosa were collected for typing. From patients with scores of isolates collected, a representative sample of strains were typed. All sites from which isolates were obtained were represented, and, in addition, all blood stream isolates were typed. The type distribution observed is summarized in Table 1. The results are compared with those observed in 1976 and 1977. It is immediately apparent that there has been an increase in the number of types recognized. Five serotypes were differentiated in 1976, 10 in 1977, and in 1977-78, 21 types were listed. However, type 4,9 was seen only in 1976, types 1,2,3,6,15, 7 and 14 only in 1977, and 13 types seen in 1976 had not previously been observed. Six types

Table 1. Serotypes of *P. aeruginosa* from Burn Patients
ISR, 1976-1978

Type	1976	Percent of Total 1977	1978
1,9		2	
1,2,3,4,9,10			7
2,3,6,15		3	
3			9
3,8,9,14			2
3,4,9,10			1
(4)8,9,11,12(14)			2
4	60(45.8) *	110(27.9)	183 (38.3)
(4),9	7		
(4)9,10	14(10.6)	20(5.0)	12 (2.5)
4,11			1
4,15			2
4,10,13			1
5			1
6		38 (7.9)	
7		3	
8		2	17
8,9			2
8,12			1
9		2	2
9,10			10
10		3	16 (3.3)
11	23(17.5)	0	35 (7.3)
12			1
14		7	
15	27(20.6)	239(60.6)	119 (24.9)
16			4
Non-reacting	0	3	21
Totals	131	394	477

* () denotes % of Total

--4,4,9,10,5,6,10, and 15 were recovered in 1977-78 in numbers significantly large. However, episodes of transmitted infection could occur even with types seen in small numbers, since not every isolate was typed, and a set of patients could be recognized as an infectious outbreak even with one strain typed per patient.

Types 4 and 15 were numerically predominant in each year. These two types, as a cause of epidemic outbreaks, obviously play a major role in the

period thus far studied.

The incidence of specific types does not reveal whether they are uniformly capable of invasive infection. Clarification of this point was sought by summarizing the serotypes of strains from wound, sputum and blood culture. The type distribution is shown in Table 2. Clearly types 4 and 15 were predominant in all categories of sources, but other types were capable of causing septicemia. six different types were recovered from blood, 12 from sputum and 16 from wounds. The recovery of *Pseudomonas* from blood was most probably a reflection of the frequency of a given type's occurrence. However, certain types showed discrepancies between sites; type 11 was found on wounds but not in blood. No explanation has, as yet, been found for this difference.

Table 2. Predominant Serotypes of *P. aeruginosa* Recovered from Blood, Wound and Respiratory Tract of Burn Patients
ISR, 1977-1978

Serotype	Source and No. of Strains Recovered		
	Blood	Wound	Sputum
3		5	2
4	14	71	54
5			1
6	2	4	18
8	1	1	17
10	1	2	11
11		16	19
12			1
15	11	46	28
16		1	3
1, 2, 3, 4, 9, 10		1	3
3, 4, 9, 14		1	1
3, 8, 9, 14	1		1
8, 9, 11, 12		1	
4, 9, 10			1
9, 10		3	5
8, 9			1

The epidemic behavior of *P. aeruginosa* types can be elucidated by sorting of patients by time intervals with the serotypes recovered at each interval. Table 3 shows the distribution of patients with the individual types by month. In August, 1977, the patient population were emerging from an acute episode of widespread *Pseudomonas* colonization. A prolonged period of infection with type 4 strains had been modified by an abrupt explosive outbreak of type 15 in the summer of 1977. In Table 3, type 4 and 15 were predominant in August 1977, but type 4 was inconspicuous thereafter until December, when this strain reappeared and continued through March. Type

Table 3. Patients Positive for Serotypes Associated with Epidemic Outbreaks of P. aeruginosa in Burn Ward, ISR

Type	Month and No. Patients Positive											
	1977			1978								
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	
1,2,3,4,9,10...	3											
4,9,10	4					3						
4	22				3	4	5	7		4		
6	5	3										
8												
9,10	3					3						
10						3						
11						3				3		
15	21					5	4	4				
				3				6	3	3		

15 dropped abruptly after August, re-emerged on a continuous basis only in March 1978. Type 6, not previously recovered, incited a separate epidemic outbreak in August and September. Type 8 caused a small outbreak in January, while types 9, 10 and 10 each occasioned two separate outbreaks. Type 11, which caused an acute outbreak in 1976, established a significant epidemic in January-March 1978. This was followed by a less intense outbreak due to type 15, which had been inconspicuous for the preceding six months.

A view of the total *Pseudomonas* population of a large burn ward is shown in Table 4, where the total bacterial population is segregated by month and serotype. The rate of colonization was not always accompanied by a transmitted outbreak, so that some types which do not appear in the total of those occasioning epidemic outbreaks still are numerically important. Types 4, 6, 9-10, 10, 11 and 15 each showed capability for persistence in successive patients that makes them potentially significant in future outbreaks.

DISCUSSION

On the basis of two years experience, typing selected strains to be sure every epidemic episode was covered, it was apparent in the 17-serum typing set includes factors for antigens that have thus far been very rare. To make the typing procedure maximally efficient, a new sequence has been evolved: Sera for types 1, 2, 3, 4, 6, 7, 8, 9, 10, 11 and 15 are run first. Positive reactions serve to categorize the strains being typed, and no further sera need be tested routinely. In the event that a series of strains exhibit a single reaction pattern, which would, of course, indicate that they constituted an epidemic strain, they should be checked with the remaining factors 5, 12, 13, 14, 16 and 17. Thus far, no reactions with factor 17 have occurred and the incidence of factors 5 and 12 has been negligible.

There is, however, a serious question as to whether the sera for types 4 and 15 may be of too broad a spectrum, so that differentiation of epidemic strains may be missed. Consideration is being given to re-evaluating these factors, with the possibility that further differentiation of strains sharing these factors can be done.

The serotyping technic has detected epidemic patterns of *Pseudomonas* infection in the burn ward, and has recognized and differentiated new epidemic types. Application of this information to more efficient control of *Pseudomonas* infection in burn wards is in view. Adaptation of the technic to a routine procedure, carried out by clinical laboratory technicians, is a feasible approach. Further study of the procedure on an experimental basis is need for achieving an effective clinically useful application of serotyping *P. aeruginosa*.

PRESENTATIONS AND/OR PUBLICATIONS - None

Table 4. Serotype Distribution by Month of P. aeruginosa in ISR Burn Patients

Type	Month and No. of Strains by Type											
	1977											
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
1,2,3,4,9,10...	3											
3,8,9,14	2											
(4),8,9,12(14)	2											
4,9,10	5		1			4	1		1			
4,15	1		1									
3	1	1				5				2		
4	83	6	1	4	3	34	18	24	3	7		
6	17	6	1	3		3	3	3		1		1
8		1				16						
8,9						1		1				
9									2			
9,10	4					4	1		1			
10	1	1			2	3	3	1	1	3	1	
11	1	2	1		1	13	5	11	1			
15	67	3	2		5	7	1	22	4	3	5	5
16						2	2					

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- PATHOGENESIS OF
BURN WOUND INFECTION: BACTERIAL FLORA OF BURN
WOUNDS OF MILITARY PERSONNEL RECEIVING SULFAMYLDON
OR SILVER SULFADIAZENE TREATMENT

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1 October 1977 - 30 September 1978

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Unclassified

ABSTRACT

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Bacterial infection is still the ultimate cause of most morbidity and death that occurs in the course of severe burns. Major species involved have lessened in number in terms of relative frequency of infection. In 1977-78, Staphylococcus aureus and Pseudomonas aeruginosa were the major offending species. Enteric forms, including Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae and Proteus mirabilis were recovered in significant numbers but were a lesser part of the whole. Former epidemic patterns of these opportunists were not seen. Detailed taxonomic effort resulted in delineation of 35 bacterial species. Most of these opportunists were not involved in a significant degree with the septic process.

Burns
Staphylococci
Enterobacteriaceae
Sepsis
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF
TROOPS WITH THERMAL INJURY -- PATHOGENESIS OF BURN
WOUND INFECTION: BACTERIAL FLORA OF BURN WOUNDS OF
MILITARY PERSONNEL RECEIVING SULFAMYLOX OR
SILVER SULFADIAZINE TREATMENT

Bacterial infection changes in burn patient populations as treatment modalities are changed, introduced or modified. However, the severity of the infection problem has not lessened, even though survival of patients with severe burns has been markedly enhanced by the development of effective topical therapy. After several years in which major burn ward epidemics of species of Enterobacteriaceae had occurred, the current report (Oct 1977- 30 Sep 1978) discloses a marked predominance of Staphylococcus aureus and of Pseudomonas aeruginosa as the predominant species. No clearcut extensive outbreak of an enteric species occurred, although Escherichia coli septicemia was more frequent than was previously the case. Continued effort at up-grading the determinative capability of the clinical microbiology section has resulted in an increase in the species differentiated in burn patients. Such precise taxonomic effort offers the only plausible route for determining the true nature of wound infection. No striking changes in infection pattern have been uncovered but the differential pattern of colonization is greatly broadened by more detailed isolation and identification.

ANTEMORTEM BACTERIOLOGY OF BURN PATIENTS - 1977-1978

The bacterial and yeast flora recovered from all clinical sources and from xenograft pigskin processed for use as a biologic dressing is summarized in Table 1. There were 223 patients on whom cultures were collected. The number of cultures per site varied; blood cultures were taken from 86% of patients cultured, while at the opposite extreme only 27.3% of patients had biopsies collected. There were 4752 blood culture bottles seeded, or 2376 blood culture sets. This represented 12.4 blood cultures per patient on whom blood cultures were collected. Seven hundred twelve isolates were recovered from blood, reflecting the high incidence of septicemia that was present during the year. In terms of total isolates, S. aureus was by far the most common organism in all sites. P. aeruginosa, with 888 isolates, was next in frequency, with E. coli, alpha hemolytic streptococci other than Group D, and K. pneumoniae the other species recovered in large numbers. Two hundred fifteen strains of Candida spp. were collected, which reflected an increase in incidence of yeast colonization - 35 bacterial species were delineated. Species notable for their absence, in view of previous years experience, included Serratia marcescens (11) strains, and Providencia stuartii, of which not one strain was recovered, in contrast to the situation 5 years ago, when 873 strains were isolated.

Total isolates are of interest in reflecting the bacterial incidence, but the frequency with which individual patients are colonized or infected is of perhaps more significance. Table 2 shows the incidence of patients positive from various sites, for each species. The sites of principal interest were

Table 1. Antemortem Bacteriology of Burn Patients 1 October 1977- 30 September 1978

Organism	Wound Surface	Blood	Source and Number of Isolates						Total
			Sputum Leukens	Urine	IV Cath. Tip	Foley Cath. Tip	Biopsy	Xeno- graft	
<i>S. aureus</i>	565	383	390	80	16	1	92	25	1552
<i>S. epidermidis</i>	39	50	55	22	1	1	3	5	176
Alpha-hemolytic									
Strep. not D	34	1	197	5	0	0	1	0	338
Non-hemolytic									
Strep. not Gp D	15	1	86	3	0	0	0	0	105
<i>S. pneumoniae</i>	2	7	36	1	0	0	0	0	46
Gp D, not enterococcus	9	3	97	0	0	0	0	0	109
Gp D enterococcus	16	15	17	9	1	1	5	1	65
Gp A Beta Streptococcus	6	1	2	0	0	0	0	0	9
Gp B Beta Streptococcus	1	4	10	0	0	0	0	0	10
Beta Streptococcus not A, B, D	3	1	28	1	0	0	0	0	33
<i>Bacillus</i> spp	7	2	2	0	0	0	3	1	15
<i>P. aeruginosa</i>	292	54	355	112	2	4	57	12	888
<i>P. cepacia</i>	1	4	3	1	0	0	0	0	9
<i>P. putida</i>	4	1	2	0	0	0	1	0	8
<i>P. fluorescens</i>	8	0	16	0	0	0	0	0	24
<i>P. stutzeri</i>	0	0	1	0	0	0	0	0	1
<i>Acinetob. antitratus</i>	1	2	3	0	0	0	0	1	7
<i>Acinetob. lwoffii</i>	1	0	0	0	0	0	0	0	1
CDC Gp. SE-2	0	1	0	0	0	0	0	0	1
<i>K. pneumoniae</i>	64	24	113	40	3	1	5	0	250
<i>K. oxytoca</i>	5	0	1	0	0	0	0	0	6
Enterocloacae	71	15	40	17	2	3	25	3	173
Enteroc. aerogenes	4	3	18	6	0	0	1	0	32
Enteroc. agglomerans	0	1	0	0	0	0	0	0	1
<i>S. marcescens</i>	2	4	5	0	0	0	0	0	11
<i>E. coli</i>	118	48	108	82	5	3	12	4	380
<i>P. mirabilis</i>	67	16	118	45	2	2	3	6	159
<i>P.morganii</i>	6	4	21	4	0	0	0	0	35
<i>P. rettgeri</i>	15	4	37	0	0	0	0	0	56
<i>P. vulgaris</i>	1	0	0	0	0	0	1	0	2
<i>Citrob. diversus</i>	1	2	2	2	0	0	1	1	9
<i>Citrob. freundii</i>	0	0	7	2	0	0	1	1	11
<i>Aeromonas hydrophila</i>	4	2	3	1	0	0	1	0	11
<i>Neisseria</i> spp.	0	0	5	5	0	0	0	0	5
<i>Corynebacterium</i> spp	4	4	3	1	0	0	0	0	12
Yeast-like organism									
<i>Candida</i> spp. by EMB Screen	58	19	35	33	0	1	12	2	158
Yeast-like organism not <i>Candida</i> spp by EMB Screen	81	19	31	36	1	2	30	15	215
<i>C. albicans</i>	45	5	43	100	0	1	5	15	202
<i>C. tropicalis</i>	7	8	8	38	0	0	1	0	62
<i>C. rugosa</i>	4	2	3	9	0	0	1	6	25
<i>C. stellatoidea</i>	8	0	0	1	0	0	0	3	12
<i>C. pseudotropicalis</i>	0	0	0	1	0	0	0	0	1
<i>C. parapsilosis</i>	0	0	0	1	0	0	0	0	1
<i>Geotrichum</i> spp.	13	2	1	0	0	0	5	0	21
<i>Flavobacterium</i> spp.	0	0	4	0	0	0	0	0	4
<i>Trichosporon</i> spp.	6	0	0	3	0	0	0	3	12
<i>Alcaligenes</i> spp.	1	0	0	0	1	0	0	0	2
<i>Listeria</i> spp.	0	0	0	0	0	0	2	0	2
No. patients cultured	178	192	126	158	39	13	61	79	
No. specimens	1251	4752	796	1079	282	14	219	415	
Total isolates	5272								
Total specimens	8808								
Total patients on whom one or more cultures were done	223								

Table 2. Antemortem Bacteriology of Burn Patients: 1 October 1977-30 September 1978

Organism	Wounds	Source and Number of Patients Positive in Culture						
		Blood	Sputum Leukens	Urine	IV Cath Tip	Foley Cath Tip	Biopsies	Xeno graft
<i>S. aureus</i>	129	84	95	46	11	1	36	25
<i>S. epidermidis</i>	32	42	41	18	1	1	3	5
Alpha-hemolytic								
Strep. no. Gp. D	26	1	98	5	0	0	1	0
Non-hemolytic								
Strep. not Gp. D	13	1	47	3	0	0	1	0
<i>S. pneumoniae</i>	2	3	31	1	0	0	0	0
Gp. D not enterococcus	9	3	54	0	0	0	0	0
Gp. D enterococcus	13	12	13	5	1	1	4	1
Gp. A Streptococcus	4	1	2	0	0	0	0	0
Gp. B Streptococcus	1	3	4	0	0	0	0	0
Beta-hemolytic Strep.								
Not A, B, D	2	1	18	1	0	0	0	0
<i>Bacillus</i> spp.	6	2	2	0	0	2	0	1
<i>P. aeruginosa</i>	82	26	61	48	2	4	23	10
<i>P. cepacia</i>	1	3	3	1	0	0	0	0
<i>P. putida</i>	4	1	1	0	0	0	1	0
<i>P. fluorescens</i>	7	0	8	0	0	0	0	0
<i>P. stutzeri</i>	0	0	1	0	0	0	0	0
<i>Acinetobacter</i> spp.								
<i>A. baumannii</i>	1	2	2	0	0	0	0	1
<i>A. lwoffii</i>	1	0	0	0	0	0	0	0
CDC Gp. SE-2	0	1	0	0	0	0	0	0
<i>K. pneumoniae</i>	34	12	49	21	3	1	5	0
<i>K. oxytoca</i>	4	0	1	0	0	0	0	0
Enterobacteriaceae	33	7	28	13	2	3	12	3
<i>Enterobacter aerogenes</i>	4	3	14	4	0	0	1	0
<i>Enterobacter agglomerans</i>	0	1	0	0	0	0	0	0
<i>S. marcescens</i>	2	2	5	0	0	0	0	0
<i>E. coli</i>	58	16	39	43	4	3	9	3
<i>P. mirabilis</i>	28	8	28	22	2	2	3	5
<i>P.morganii</i>	5	3	12	4	0	0	0	0
<i>P. rettgeri</i>	4	3	9	0	0	0	0	0
<i>P. vulgaris</i>	1	0	0	0	0	0	1	0
<i>Citrobacter diversus</i>	1	1	2	2	0	0	1	1
<i>Citrobacter freundii</i>	0	0	7	2	0	0	1	1
<i>Aeromonas hydrophila</i>	4	0	3	1	0	0	1	0
<i>Neisseria</i> spp.	0	0	4	0	0	0	0	0
<i>Corynebacterium</i> spp.	4	4	2	1	0	0	0	0
Yeast-like organism								
<i>Candida</i> spp. by EMB Screen	32	10	25	31	0	1	9	2
Yeast-like organism not <i>Candida</i> spp. by EMB Screen	39	14	16	26	1	1	12	13
<i>C. albicans</i>	26	4	23	19	0	1	4	3
<i>C. tropicalis</i>	5	5	8	9	0	0	1	0
<i>C. rugosa</i>	3	2	2	2	0	0	1	4
<i>C. stellatoidea</i>	4	0	0	1	0	0	0	3
<i>C. pseudotropicalis</i>	0	0	0	1	0	0	0	0
<i>C. parapsilosis</i>	0	0	0	1	0	0	0	0
<i>Geotrichum</i> spp.	7	2	1	0	0	0	5	0
<i>Flavobacterium</i> spp.	0	0	3	0	0	0	0	0
<i>Trichosporium</i> spp.	4	0	0	0	0	0	0	1
<i>Alcaligenes</i> spp.	1	0	0	0	0	0	0	0
<i>Listeria</i> spp.	0	0	0	0	0	0	1	0
Total No. Patients Sampled	178	192	126	158	39	13	61	79

wound, biopsy, blood and sputum. Major species represented here were S. aureus, P. aeruginosa, E. coli, K. pneumoniae and, from past experience, Enter. cloacae. The proportion of patients positive for these species is shown in Table 3. Staphylococci were consistently the predominant species in all sites. Pseudomonas strains were relatively more frequent on wound surface than in biopsy, suggesting that the colonizing potential greatly exceeded the invasive capability. The enteric species were relatively infrequent in blood, in contrast to the incidence in sputum, wounds and biopsies.

BURN WOUND BACTERIOLOGY

The source of sepsis in severely burned patient has not been established conclusively but it is most often ascribed to the wound flora itself. The exception is, of course, Pseudomonas burn wound sepsis, in which a characteristic lesion is demonstrable. It is axiomatic that wound flora is modified by the specific therapeutic regimen that is employed, and in the Institute of Surgical Research, wound treatment with Sulfamylon^R and silver-sulfadiazine burn creams, and with 5% Sulfamylon soaks, is routine.

The significant part of the burn surface wound flora for this reporting period is shown in Table 4. Eight species, out of the 35 bacterial species listed in Table 1, are shown; no other species appeared in numbers sufficient to justify its inclusion. S. aureus was vastly the preponderant form, in keeping with its role as the major pathogen in current burns wounds. Staphylococcus epidermidis was relatively frequent as an occasional contaminant, but was not consistently present in any patient. This applies also to the alpha-streptococci. P. aeruginosa, although it played a major role in wound infection, involved only 46% of the patients cultured. The coliform bacteria were all present in rates from 15% to 19% of patients, except for E. coli, recovered in wounds of 33% of patients cultured. Sepsis due to Enterobacteriaceae decreased significantly in the year reported.

A species which is rare in wounds, but continually sought since its presence is associated with severe consequences is the group A streptococcus (Streptococcus pyogenes). Four patients were found to harbor this species in 1977-1978. Total isolates from burn wounds have fluctuated; in 1969, 8 strains were recovered; in 1970, 2; 1971, 1; 1972, 56; 1973, 3; 1974, 30; 1975, 3, 1976, 0, and 1977-1978, 6.

RESPIRATORY TRACT FLORA IN BURNS

Pneumonia remains a major complication in severe burns, and the flora of sputum and Luken's tube remains the principal indicator of the specific infection problem. Table 5 shows the principal species in sputum cultures. Once again, the major species was S. aureus, S. epidermidis, alpha-streptococci and Group D streptococci were numerous but not exceptional in sputum. A new finding was the presence of Streptococcus pneumoniae in one-fourth of the patients cultured. This organism is being watched carefully, since pneumococcal pneumonia could be a major concern in these patients. P. aeruginosa was the most common gram-negative species with K. pneumoniae

Table 3. Percentage of Patients Cultured at a Given Site and Positive for Each Species

Species	Source and % of Patients Positive			Situation
	Wound	Biopsy	Blood	
<i>S. aureus</i>	72.1	59.0	43.7	75.3
<i>P. aeruginosa</i>	46.0	37.1	13.5	48.4
<i>E. coli</i>	32.5	14.7	8.3	30.9
<i>K. pneumoniae</i>	19.1	8.1	6.2	38.8
<i>Enterocloacae</i>	18.5	19.6	3.6	22.2

Table 4. Burn Wound Surface Flora in 178 Patients: 1 October 1977- 30 September 1978

Species	No. of Strains	No. of Patients Positive	% of Cultured Patients Positive
<i>S. aureus</i>	565	129	72.5
<i>S. epidermidis</i>	39	32	18.0
<u>Alpha-Strep.</u> *	34	26	14.6
<i>P. aeruginosa</i>	292	82	46.1
<i>K. pneumonia</i>	64	34	19.1
<i>Enterocloacae</i>	71	33	18.5
<i>E. coli</i>	118	58	32.6
<i>P. mirabilis</i>	67	28	15.7

* These strains were other than Group D.

the most numerous enteric species. *E. coli* outnumbered the formerly critical species, *Enterocloacae*. The relative distribution of species has not varied markedly in the past year.

SEPTICEMIA IN BURNED PATIENTS

The critical aspect of bacterial involvement of burned wounds is the

Table 5. Principal Species of Bacteria Recovered
from Respiratory Tract of 126 Burned Patients:
1 October 1977 - 30 September 1978

Species	No. of Isolates	No. of Patients Positive	% of Cultured Patients Positive
<i>S. aureus</i>	350	95	75.4
<i>S. epidermidis</i>	55	41	32.5
Alpha-Strep. not Gp D	297	98	77.8
<i>S. pneumoniae</i>	36	31	24.6
Strep. Gp.D	114	67	52.9
<i>P. aeruginosa</i>	355	61	48.4
<i>K. pneumoniae</i>	113	49	38.9
Enterocloacae	40	28	22.2
<i>E. coli</i>	108	39	31.0
<i>P. mirabilis</i>	118	28	22.2

incidence of bacteremia or septicemia. In 1977-1978, 192 patients had one or more blood cultures drawn. Of these, 116 (60.4%) had at least one positive culture. In contrast to other seeded sites, such as wound and respiratory tract, the capability for blood stream invasion is too important to be noted only with predominant species. Table 6 presents the positive blood culture results for this year. The recovery of species not usually regarded as pathogenic continued. *S. epidermidis* was prominent in recovery, but few patients had more than one culture positive. In the rate of cultured patients positive, it is to be borne in mind that many patients had blood cultures drawn on a routine basis. The rate of positive blood cultures out of all patients cultured is relatively constant. In 1977, 58% of all patients cultured had a positive blood. Only *S. aureus* and *P. aeruginosa* played a major part in causing septicemia. Enteric species, including *Klebsiella* and *E. coli*, were in only a small part of the total of positives.

Most of the bacteremia encompassed in this summary were mixed infections, in which different species would appear in succession, or in some instances, in mixed infections. There were 50 patients in whom only one species of bacteria was recovered. Table 7 presents this information. When placed in contrast to the overall blood culture recovery, it is apparent that only *S. aureus*, among pathogenic species, appeared as the sole species in a significant number of cases. The incidence of one-species infections with, for example, *P. aeruginosa*, was minimal. The overall pattern is one of consistent mixed infection.

Table 6. Blood Culture Isolates from 192 Burned Patients:
1977-1978

Species	Total No. of Isolates	No. of Patients Positive	% of Cultured Patients Positive
<u>S. aureus</u>	383	84	43.7
<u>S. epidermidis</u>	50	42	21.9
Alpha-hemolytic Strep. not GpD	1	1	0.5
Non-hemolytic Strep. not GpD	1	1	0.5
S. pneumoniae	7	3	1.6
Gp. D not an enterococcus	3	3	1.6
Gp. D enterococcus	15	12	6.3
Gp. A beta streptococcus	1	1	0.5
Gp. B beta streptococcus	4	3	1.6
Beta-hemolytic Strep. not Gp. A, B, D	1	1	0.5
Bacillus spp.	2	2	1.0
<u>P. aeruginosa</u>	54	26	13.5
<u>P. putida</u>	1	1	0.5
P. cepacia	4	3	1.6
<u>K. pneumonia</u>	24	12	6.3
Enteroc. cloacae	15	7	3.6
Enteroc. aerogenes	3	3	1.6
Enteroc. agglomerans	1	1	0.5
S. marcescens	4	2	1.0
<u>E. coli</u>	48	16	8.3
P. mirabilis	16	8	4.2
P. morganii	4	3	1.6
P. rettgeri	4	3	1.6
Citrob. diversus	2	1	0.5
Aeromonas hydrophila	2	1	0.5
Acinetob. anitratum	2	2	1.0
Micrococcus spp.	1	1	0.5
P. maltophilia	1	1	0.5
CDC Gp. VE-2	1	1	0.5
Yeast-like organism:			
Candida spp. by EMB Screen	19	10	5.2
Yeast-like organism not			
Candida spp. by EMB Screen	19	14	7.3
C. albicans	5	4	2.1
C. tropicalis	8	5	2.6
C. rugosa	2	2	1.0
Geotrichum spp.	2	2	1.0
Corynebacterium spp.	4	4	2.1

Underlined species represent numerically important organisms.

Table 7. Bacteremia with Only One Species of Bacteria
Recovered: Burn Patients - 1 October 1977- 30 September 1978

Species	No. Patients with One Species Recovered	Ave. No. of Positive Blood Cultures/ Patient	No. Deaths	% Mortality for One Species Bacteremia
<i>S. aureus</i>	27	2.1	9	33.3
<i>S. epidermidis</i>	14	1	2	14.3
<i>Strep. pneumoniae</i>	2	1	1	50.0
Gp D enterococcus	1	1	0	0
Gp D not enterococcus	1	1	1	100
<i>Aeromonas hydrophila</i>	1	1	1	100.
<i>Entero. aerogenes</i>	1	1	1	100.
<i>P. aeruginosa</i>	1	1	1	100
<i>C. albicans</i>	1	1	0	0
<i>Corynebacterium</i> spp.	1	1	0	0

The mixed bacteremia picture is presented in Table 8. As in previous years, the mixture is very heterogeneous; there was no plausible sequence of bacteremic species, except for the impression that *S. aureus* tended to be the first species recovered. The basic fact is that this succession of infections makes the concept of immunization against *P. aeruginosa* in such a population of relatively little meaning.

BURN INFECTION AND BIOPSIES

Wound biopsy as a major feature of burn wound monitoring has become widely accepted. Table 9 presents the results of 219 biopsy specimens collected from 61 patients in the reporting period. The relative frequency of species recovered, and the mortality rate associated with each species is shown. When compared with wound surface cultures, as in Table 2, a striking drop in species recovered and frequency of recovery is seen. It was evident that only *S. aureus*, *P. aeruginosa*, *Entero. cloacae* were recovered in tissues more often than in one patient out of 5. Opportunistic colonizing species, such as, *S. epidermidis*, alpha streptococci, *K. pneumoniae* and *E. coli*, were infrequent in sub-surface tissue. Seventeen species were recovered, but only those listed were in numbers sufficient to permit their consideration as transmission or cross-contamination indicators. A fatal outcome was associated more frequently with the enteric species than with *Pseudomonas* presence,

Table 8. Blood Culture Isolates in Patients with More than One Species Recovered
1 October 1977 - 30 September 1978

Species	No. of Patients
<i>S. aureus</i> , <i>S. epidermidis</i>	2
<i>S. aureus</i> , <i>Strep. pneumoniae</i>	1
<i>S. aureus</i> , Group D enterococcus	2
<i>S. aureus</i> , <i>P. aeruginosa</i>	4
<i>S. aureus</i> , <i>P. maltophilia</i>	1
<i>S. aureus</i> , <i>S. marcescens</i>	1
<i>S. aureus</i> , <i>Corynebacterium</i> spp.	1
<i>S. aureus</i> , <i>K. pneumoniae</i>	1
<i>S. aureus</i> , Yeast-like organism not <i>Candida</i> spp by EMB Screen	1
<i>S. aureus</i> , Group B Beta-hemolytic streptococcus	2
<i>S. epidermidis</i> , <i>C. rugosa</i>	1
<i>P. aeruginosa</i> , <i>S. marcescens</i>	1
<i>K. pneumoniae</i> , <i>P. mirabilis</i>	3
<i>K. pneumoniae</i> , Group D enterococcus	2
<i>K. pneumoniae</i> , <i>P. rettgeri</i>	1
Enterocloacae, <i>Citro. diversus</i>	1
Enterocloacae, Enterocloacae, <i>aerogenes</i>	1
<i>E. coli</i> , <i>P. mirabilis</i>	1
<i>P. mirabilis</i> , Group D enterococcus	1
Yeast-like organism <i>Candida</i> spp by EMB Screen, Yeast-like organism not <i>Candida</i> spp by EMB Screen	1
Yeast-like organism not <i>Candida</i> spp by EMB Screen, <i>C. albicans</i>	1
<i>S. aureus</i> , Group D enterococcus, <i>Strep. pneumoniae</i>	1
<i>S. aureus</i> , <i>P. aeruginosa</i> , Yeast-like organism not <i>Candida</i> spp by EMB Screen	1
<i>S. aureus</i> , Group A Beta-hemolytic <i>Strep.</i> , Group D not enterococcus	1
Number of patients with 2 or more species recovered	22
Number of patients with 2 species recovered	22
Number of patients with 3 species recovered	3
Number of patients with 4 species recovered	0
Number of patients with:	
<i>S. aureus</i>	15
<i>P. aeruginosa</i>	6
<i>K. pneumoniae</i>	5
Group D enterococcus	5
<i>P. mirabilis</i>	4
<i>S. epidermidis</i>	3
Yeast-like organism not <i>Candida</i> spp by EMB Screen	3
Group D not an enterococcus	2
Group B Beta-hemolytic Streptococcus	2
Enterocloacae	2
<i>P. maltophilia</i>	1
<i>E. coli</i>	1
<i>P. rettgeri</i>	1
<i>Strep. pneumoniae</i>	1
<i>S. marcescens</i>	1
Group A Beta-hemolytic Streptococcus	1
Yeast-like organism <i>Candida</i> spp. by EMB Screen	1
<i>C. albicans</i>	1
Enterocloacae, <i>aerogenes</i>	1
<i>Citro. diversus</i>	1
<i>Candida rugosa</i>	1
<i>Corynebacterium</i> spp	1

Table 9. Bacterial Flora of Biopsies of Burn Wounds of 61 Patients: 1 Oct. 77 - 30 Sep. 78

Species	No. of Patients Positive	% of Patients Positive	No. of Patients with Positive Cultures who Expired	% of Patients who Expired
<i>S. aureus</i>	36	59.0	20	55.5
<i>S. epidermidis</i>	3	4.9	1	33.3
Alpha-hemolytic Strep. not Cp. D	1	1.6	1	100
Non-hemolytic Strep. not Cp. D	1	1.6	1	100
Group D enterococcus	4	6.6	1	25.0
<i>Bacillus</i> spp.	2	3.3	1	50.0
<i>P. aeruginosa</i>	23	37.7	8	34.8
<i>P. putida</i>	1	1.6	0	0.0
<i>K. pneumoniae</i>	5	8.2	3	60.0
<i>Enterocloacae</i>	12	19.7	11	91.7
<i>Enteroc. aerogenes</i>	1	1.6	1	100
<i>E. coli</i>	9	14.8	6	66.6
<i>P. mirabilis</i>	3	4.9	1	33.3
<i>P. vulgaris</i>	1	1.6	1	100
<i>Citro. diversus</i>	1	1.6	1	100
<i>Citro. freundii</i>	1	1.6	1	100
<i>Aeromonas hydrophila</i>	1	1.6	1	100
Yeast-like organism Candida spp by EMB Screen	9	14.8	9	100
Yeast-like organism not Candida spp by EMB Screen	12	19.7	8	66.6
<i>C. albicans</i>	4	6.6	4	100
<i>C. rugosa</i>	1	1.6	1	100
<i>C. tropicalis</i>	1	1.6	1	100
<i>Geotrichum</i> spp.	5	8.2	5	100
<i>Listeria</i> spp.	1	1.6	1	100
Number of Specimens: 219	Number of Specimens per patient (ave.): 3.6			

in contrast to observations of previous years. The presence of gram-negative bacilli in deep eschar or sub-eschar tissue is a plausible reason for regarding the patient as at great risk.

CATHETER TIP CULTURES

Infection of veins as a result of indwelling catheters is a matter of continued concern in severely burned patients. With wider use of hyperalimentation, such prolonged catheterization has become more common. A review of culture results on 282 specimens from 39 patients as shown in Table 10 showed that there was a decrease in incidence of bacterial colonization on these tips, in contrast to results observed in previous years. The 8 bacterial species recovered included only one S. aureus in more than one-fourth of the patients sampled. The remaining species were recovered in not more than 4 patients (E. coli). The small incidence of recoveries suggests increasing success in the aseptic maintenance of intravenous catheter tips.

Table 10. Bacterial Flora of IV Catheter Tips:
1 October 1977 - 30 September 1978

Organism	No. of Isolates	No. of Patients Positive	% Total Patients Positive
<u>S. aureus</u>	16	11	28.2
<u>S. epidermidis</u>	1	1	2.6
Group D enterococcus	1	1	2.6
<u>P. aeruginosa</u>	2	2	5.1
<u>K. pneumoniae</u>	3	3	7.7
<u>Enterocloacae</u>	2	2	5.1
<u>E. coli</u>	5	4	10.3
<u>P. mirabilis</u>	2	2	5.1
Yeast-like organism not Candida spp. by EMB Screen	1	1	2.6

No. of Patients Cultured: 158; No. of Cultures: 1079

URINARY TRACT BACTERIAL FLORA

Urine cultures were collected on 158 out of 242 patients admitted. The recovery of bacterial species is shown in Table 11. The overall distribution resembles that seen in surface and sputum cultures. S. aureus, P. aeruginosa, E. coli and K. pneumoniae were the most common species. Seventeen bacterial species were recovered. The sequence of events and

Table 11. Urine Cultures: 1 October 1977 - 30 September 1978

Organism	No. of Isolates	No. of Patients Positive	% Total Patients Positive
<i>S. aureus</i>	89	46	29.1
<i>S. epidermidis</i>	22	18	11.5
Alpha-hemolytic Strep. not Gp. D	5	5	3.2
Non-hemolytic Strep. not Gp. D	3	3	1.9
<i>S. pneumoniae</i>	1	1	0.6
Gp. D enterococcus	9	5	3.2
Beta hemolytic Strep. not Gp. A, B, D	1	1	0.6
<i>P. aeruginosa</i>	112	448	30.8
<i>P. cepacia</i>	1	1	0.6
<i>K. pneumoniae</i>	40	21	13.5
Enteroc. cloacae	17	13	8.3
Enteroc. aerogenes	6	4	2.6
<i>E. coli</i>	82	43	27.6
<i>P. morabillis</i>	45	22	14.1
<i>P. morganii</i>	4	4	2.6
<i>Citro. diversus</i>	2	2	1.3
<i>Citro. freundii</i>	2	2	1.3
Yeast-like organism Candida spp. by EMB Screen	33	31	19.9
Yeast-like organism not Candida spp. by EMB Screen	36	26	16.7
<i>C. albicans</i>	100	19	12.2
<i>C. tropicalis</i>	38	9	5.8
<i>C. pseudotropicalis</i>	1	1	0.6
<i>C. rugosa</i>	9	2	1.3
<i>C. parapsilosis</i>	1	1	0.6
<i>C. stellatoidea</i>	1	1	0.6
<i>Trichosporon pencillatum</i>	3	3	1.9
<i>Aeromonas hydrophila</i>	1	1	0.6
<i>Corynebacterium</i> spp	1	1	0.6

Number of Patients Cultured: 158; Number of Cultures: 1079

species distribution suggest that urinary tract infection is essentially a reflection of the wound and sputum flora, rather than being a primary infection site from which infection extends to initiate sepsis.

PORCINE XENOGRAPH CULTURES

The extensive use of xenograft as a biologic dressing has prompted more systematic culturing of this material. Samples were taken from xeno-

graft sheets at the time of their preparation for use, and designated for the patient on whom they were to be used.

Table 12 summarizes the results of these observations. The distribution of species resembles that seen in the burn wound flora. Although the initial impression is that of heavily contaminated xenograft, these results must be interpreted with caution. Since the culture was taken at the time of xenografting, there is a strong possibility that the sample was contaminated at the time of collection. The contaminants described in porcine skin have been primarily *Bacillus* spp. and micrococci which were not conspicuous in this series. The positive growth shown in this study is included because there is every reason to believe the xenograft, indeed, harbored these organisms. But it remains a strong possibility that many of them were introduced at the time the samples were removed from the prepared xenograft before the material was applied to the wound.

Table 12. Xenograft (Porcine) Cultures: 1 October 1977 - 30 September 1978

Organism	No. of Isolates	No. of Patients Positive	% Total Patients Positive
<i>S. aureus</i>	56	25	31.6
<i>S. epidermidis</i>	5	5	6.3
<i>Gp D enterococcus</i>	1	1	1.3
<i>Bacillus</i> spp	1	1	1.3
<i>P. aeruginosa</i>	12	10	12.6
<i>Enteroc. cloacae</i>	3	3	3.7
<i>E. coli</i>	4	3	3.7
<i>P. mirabilis</i>	6	5	6.3
<i>Citro. diversus</i>	1	1	1.3
<i>Citro. freundii</i>	1	1	1.3
Yeast-like organism <i>Candida</i> spp. by EMB Screen	2	2	2.5
Yeast-like organism not <i>Candida</i> spp. by EMB Screen	15	13	16.5
<i>C. albicans</i>	3	3	3.8
<i>C. stellatoidea</i>	3	3	3.8
<i>C. rugosa</i>	6	4	5.1
<i>Trichosporon pencillatum</i>	3	1	1.3
<i>Acinetobacter antitratum</i>	1	1	1.3
Number of Patients Cultured: 79; Number of Cultures: 415			

POSTMORTEM BACTERIOLOGY

The bacterial flora at autopsy has been regarded as part of the more definitive information as to nature of the fatal infection. In this year, 62 autopsies were performed of which 60 involved tissues submitted for culture.

The number of isolates and sources are shown in Table 13. There were 25 species recovered. Numerically predominant bacteria were *S. aureus*, *P. aeruginosa*, *E. coli* and *Enterocloacae*. In tissue localization, 11 species were recovered from liver, 12 from spleen, 21 from lung tissue and 15 from wound samples. Liver and spleen offered the most convincing reflection of the identity of sepsis causation, and the wound samples closely resembled these viscera in bacterial distribution. The lung tissues harbored more species that were not seen elsewhere and may be regarded as probable contaminants rather than etiologic agents.

Table 13. Postmortem Bacteriology of Burn Patients:
1 October 1977 - 30 September 1978

Organism	Recovered at Autopsy	Liver	Spleen	Lung	Wound	Blood
<i>S. aureus</i>	240	25	19	105	82	9
<i>S. epidermidis</i>	5	0	0	4	1	0
Alpha-hemolytic Strep. not Gp. D	3	1	0	1	0	1
Non-hemolytic Strep. not Gp. D	10	0	1	6	3	0
Gp. D. not enterococcus	4	2	0	2	0	0
Gp. D enterococcus	18	5	1	7	4	1
<i>Strep. pneumoniae</i>	2	0	0	2	0	0
<i>Bacillus</i> spp.	1	0	0	0	1	0
<i>P. aeruginosa</i>	114	6	8	50	45	5
<i>P. alcaligenes</i>	2	0	0	2	0	0
<i>P. cepacia</i>	2	0	0	1	1	0
<i>P. putida</i>	1	0	0	1	0	0
<i>P. maltophilia</i>	2	0	0	1	1	0
<i>Acinetobacter lwoffii</i>	6	1	4	1	0	0
<i>Acinetobacter antitratum</i>	12	1	1	3	6	1
<i>K. pneumoniae</i>	21	4	3	8	4	2
<i>Enterocloacae</i>	28	3	2	15	7	1
<i>Enterococcus aerogenes</i>	1	0	1	0	0	0
<i>E. coli</i>	62	8	9	31	6	8
<i>P. mirabilis</i>	41	5	5	19	4	8
<i>P. morganii</i>	7	0	1	4	0	2
<i>P. rettgeri</i>	2	0	0	2	0	0
<i>P. vulgaris</i>	1	0	0	1	0	0
<i>Citrobacter diversus</i>	1	0	0	0	1	0
<i>Aeromonas hydrophila</i>	1	0	0	0	1	0
Yeast-like organism <i>Candida</i> Spp. by EMB Screen	34	1	2	10	21	0
Yeast-like organism not <i>Candida</i> spp. by EMB Screen	32	1	1	3	26	1
<i>C. albicans</i>	5	0	0	1	4	0
<i>C. rugosa</i>	2	0	0	1	1	0
<i>Corynebacterium</i> spp.	7	0	2	3	2	0
CDC Gp. 5E-1	1	0	0	0	1	0
Number of Patients Autopsied:	62					

PPUBLICATIONS - None

PRESENTATIONS

R.B. Lindberg. Lecture - University of Heidelberg School of Medicine II:
"Erweiterte Lehr-und Fortbildungs veranstalt -ung: 15 Sep 78 - "Problems
of Mixed Infections in Burns and Relation to Monoinfections in Septicemia".

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- THE ROLE OF FUNGI
IN BURN WOUND INFECTION: OBSERVATIONS ON BIOPSY
AND AUTOPSY TISSUES FROM SERIOUSLY BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 October 1977 - 30 September 1978

Investigators:

Robert B. Lindberg, PhD
Jack R. Henderson, PhD
Albert T. McManus, Jr, Captain, MSC
William S. Hardy, SP5

Reports Control Symbol MEDDH-288 (R1)

Unclassified

ABSTRACT

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US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

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Autopsy and biopsy tissues were cultured for fungi. Fungal colonization was not epidemic; the majority of strains were *Candida* spp. Eight genera of fungi were recovered in biopsy tissues, and 10 from autopsies. Viscera harbored as extensive a flora as did the burn wound. Aspergillus spp. was the predominant genus.

Fungi
Mucor
Aspergillus
Phycomycosis
Burns
Human

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- THE ROLE OF FUNGI IN BURN WOUND INFECTION: OBSERVATIONS ON BIOPSY AND AUTOPSY TISSUES FROM SERIOUSLY BURNED SOLDIERS

The nature of fungal infection in burns is not yet clearly understood. Severe, fulminating infections appear in the burn population at irregular intervals, with little evidence that infection is spread by cross-contamination. Yet, strains of fungi identical to those recovered from fungal wound sepsis are often recovered from the air and environment of a burn patient. In the current reporting period, relatively little colonization occurred by fungi, to judge from the rate of recovery from tissue specimens. Fungi were recovered in one-third of patients admitted to the burn unit during the period 1973-1977. This includes, however, a vast predominance of yeasts, predominantly *Candida* spp. Only 83 of 1245 patients admitted developed invasive infection, as evinced by invasion of viable tissue by fungal elements or by recovery of yeasts in blood culture. The latter event does not of necessity presage a life-threatening episode of sepsis; yeast bacteremia can occur with no specific indications of clinical illness.

FUNGI IN BIOPSY SPECIMENS

Sixty-one patients had 219 biopsy specimens collected; each specimen was cultured for fungi on Sabouraud's agar. Eight genera, including *Candida*, were identified (Table 1). The recovery of fungi from wounds has shown a continuous diminution in the past 5 years. There is no obvious technical explanation for this discrepancy. The techniques have not been changed, and burn patients have been exposed to both Sulfamylon^R and silver-sulfadiazine during this time.

Table 1. Fungi Recovered from Biopsies - 1977-1978

Genus	No. Patients Positive	No. of Strains Recovered
<i>Aspergillus</i> spp.	10	21
<i>Aspergillus niger</i>	1	2
<i>Fusarium</i> spp	3	4
<i>Cephalosporium</i> spp	5	5
<i>Alternaria</i> spp	4	6
<i>Mucor</i> spp.	2	4
Yeast-like organisms	10	18
<i>Candida albicans</i>	2	2
<i>Geotrichum</i> spp.	7	10

The lower incidence of fungi in wounds began before silver-sulfadiazine was introduced in the burn ward. The comparison of fungal re-

coveries in biopsy is shown in Table 2. The smaller number of patients on whom biopsies were done indicates a relatively higher incidence in burn patients than in recent years.

Table 2. Comparison of Recovery of Fungi from Biopsies of Burn Wounds - 1972-1978

Genus	Year and Number of Strains Recovered					
	1972	1973	1974	1975	1976-7	1977-8
Aspergillus	11	17	5	2	5	23
Cephalosporium	15	5	5	1	4	5
Fusarium	33	23	17	2	4	4
Sepedonium	0	1	0	0	3	0
Penicillium	1	1	3	0	1	0
Alternaria	7	2	3	1	3	6
Trechophyton	0	0	0	0	1	0
Mucor	2	2	0	0	1	4
Rhizopus	3	2	0	0	0	0
Curvularium	3	2	3	0	0	0
Scopulariopsis	11	0	0	0	0	0
Diplosporium	1	0	0	0	0	0
Helminthosporium	0	9	2	0	0	0
Geotrichum	1	0	4	0	0	10
Candida spp	46	141	144	15	22	20
No. of Patients Cultured	201	106	135	63	113	61
No. Genera	12	11	9	5	9	7

FUNGI RECOVERED AT AUTOPSY

As with biopsies, autopsy tissues, including liver, spleen, lung and burn wound, are routinely cultured for fungi. Although the wound, due to its surface exposure, is usually thought of as being far more likely to acquire fungus colonization than are visceral sites, the actual intensity of colonization is comparable. The implication that such organ localization is hematogenous, originating in the wound, has not been substantiated. Table 3 presents the results from 86 autopsies on which cultures were carried out. Seven species were recovered from wounds and from viscera, but they were not identical. Four genera occurred in both viscera and wound: these were Aspergillus, Cephalosporium, alternaria and Candida sp. Three were found only in wounds - Fusarium, Helminthosporium, and Candida albicans. Two were found only in viscera - Penicillium and Nigrosporium. The most common fungus was Aspergillus. No others were present in large numbers. Phycomycetes were not found in any autopsy samples. Despite tissue diagnosis of mucormycosis, samples did not grow out Mucor. These patients were, however, positive for Mucor on biopsy.

Table 3. Fungi Recovered from Viscera and
Burn Wounds at Autopsy - 1977-1978

Genus	Wounds		Viscera	
	Patients Positive	No. of Strains	Patients Positive	No. of Strains
Aspergillus	8	14	8	11
Fusarium	1	1	0	0
Cephalosporium	2	2	3	5
Alternaria	5	5	3	4
Penicillium	0	0	2	2
Helminthosporium	2	2	0	0
Nigrosporium	0	0	1	1
Geotrichium	0	0	5	7
Candida spp	8	14	8	14
C. albicans	1	2	0	0

Continued monitoring of burn patients for fungi from biopsies and/or autopsies is essential if the confirmation of identity is to be achieved. The saprophytic nature of several of the genera recovered was notable.

PRESENTATIONS/PUBLICATIONS - None

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- DETECTION OF
ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

Robert B. Lindberg, PhD
Albert T. McManus, Captain, MSC
Virginia C. English, MS

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

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To obtain more specific information regarding the relationship of endotoxin formation to virulence of strains of Pseudomonas aeruginosa in burned rats, a technic was developed for measuring endotoxin produced in a synthetic substrate. An optimal formulation was found to be tissue culture substrate L-15, without serum. Consistent growth rates and well defined endotoxin production rates can be measured in this system.

Pseudomonas
Burns
Endotoxin
Sepsis
Virulence
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- DETECTION OF ENDOTOXIN
IN BURNED SOLDIERS WITH SEPSIS

The detection of minute amounts of endotoxin in biologic fluids and tissues, through its attribute of inciting coagulation of a lysate of the amoebocytes of Limulus polyphemus has opened the path for extensive studies of endotoxin and its relation to the pathogenesis of infection and sepsis. Studies in this laboratory (1,2) have shown that circulating endotoxin and bacteremia are not correlated. Endotoxemia and positive blood cultures occurred together only with random frequency. Further, demonstration of endotoxin in blood was not predictive of subsequent septicemia.

These observations do not negate the potential significance of endotoxin as a major factor in gram negative shock. The anomalous results have, however, stimulated the search for explanations of the role of endotoxin as it relates to pathogenicity or virulence of invasive gram negative bacteria in burn injury. In any study of an infectious process, an animal model greatly facilitates the investigation, and in the case of burn injury and infection, the ISR burned, infected rat model has been invaluable. Virulent strains of Pseudomonas aeruginosa are used, with known lethal potential. In studying such virulence, a large number of patient strains were tested, and it was found that half of these were virulent in varying degrees, as contrasted to the 100% virulent and avirulent strains. A potentially significant variable relating to these differences in virulence is the amount of endotoxin elaborated by individual strains, and its rate of production.

As assessment of such variations in endotoxin production was proposed, and it was apparent that a technic was needed in which endotoxin production would be a function of bacterial growth, free from presence of preformed endotoxin in the culture substrate. Synthetic media were prepared and tested to develop such a substrate. Simple formulations with endotoxin-free carbon and nitrogen sources supported growth, but growth rates were too often slow and variable. A more effective base for determination of endotoxin-producing capability was found to be one of the commercially available tissue culture fluids. Out of the readily available formulations, medium L-15, prepared by BBL (Division of Bioquest), was found to furnish rapid, reproducible growth from small inocula of a wide range of Pseudomonas strains. This formulation containing 17 amino acids, B complex vitamin components, and inorganic components furnished a physiologically balanced solution. The filtrate and cellular components could be assessed for free and cell-bound endotoxin. The technic makes assessment of endotoxin-producing capability possible. The

1. Lindberg RB, English VC, Pruitt BA, Jr, Mason AD, Jr: Detection of endotoxin in burned soldiers with sepsis. USA Inst Surg Res Annual Rpt FY 1973. BAMC, Fort Sam Houston, Texas. Section 6.

2. McManus AT, Jr, English VC, Lindberg RB: Detection of endotoxin in burned soldiers with sepsis. USA Inst Surg Res Annual Rpt FY 1977, BAMC, Fort Sam Houston, Texas. p. 295.

relationship of this attribute to virulence, invasiveness, and possibly to immunogenicity can now be further studied.

PUBLICATIONS

McManus AT, Jr, Linberg RB, Pruitt BA, Jr, Mason AD, Jr: Endotoxemia and septicemia in the burned patient. Fed Proc 37: 432, 1978.

PRESENTATIONS - None

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- EMERGENCE OF
METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS TYPE
84 IN BURNED MILITARY PERSONNEL

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Epidemic staphylococcus infection in the Institute of Surgical Research burn wards followed a previously observed trend to establish a predominant type 84,85 epidemic with 10 additional types observed only in negligible numbers. Blood stream infections yielded only type 84,85 among typable strains; unlike previous observations, no other type was recovered. Methicillin resistance had previously been shown to shift over long periods; in this year, the staphylococcus population shifted from resistant to sensitive in a few weeks. After three months, resistant strains reappeared, and the cycle was repeated. Oxacillin-resistance was for six months the opposite of methicillin-resistance and nafcillin was entirely ineffective for the whole period. Other antibiotics were, in general, not linked in this oscillating pattern to methicillin; only with clindamycin was an abrupt shift from sensitivity to resistance observed.

Staphylococcus
Burns
Septicemia
Burn infections
Phage type
Infections
Antibiotic

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- EMERGENCE OF
METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS TYPE 84
IN BURNED MILITARY PERSONNEL

Antibiotic resistance has, in the decades since these agents appeared, tended to increase, with passage of time, across the whole spectrum of target bacterial species. This phenomenon has been of especial import with staphylococci. Staphylococcus aureus in tissue infections and in staphylococcemia has increased in incidence in the ISR burn patients in the past two years. In 1977-1978, 54% of 640 strains recovered in blood culture were staphylococci, while the next most frequently encountered species, Pseudomonas aeruginosa, made up only 11% of the septicemic strains. Evidence of increasing antibiotic resistance of staphylococci thus pertains to a numerically important pathogen and indeed such resistance may constitute one cause of increasing incidence of staphylococci in the burn ward. Strain identification of this infecting population by phage typing has aided greatly in recognition of the monotype nature of these hospital epidemics.

PHAGE TYPES OF STAPHYLOCOCCUS AUREUS, 1977-1978

Staphylococcus strains, differentiated by phage type have for over a decade fallen into a pattern of predominance of a small number, or even a single type, associated with a peripheral population of varying types, none of which was numerous. Since 1971, the staphylococcus population has varied between a virtually pure type 84 epidemic to a preponderance of type 84,85. At all times, a significant number of strains have been nontypable; this category in most years made up from 16% to 20% of the total of strains collected.

The distribution of phage types in 1977-1978 is shown in Table 1. This includes strains from clinical material and from post-mortem material. The preponderance of typable strains were type 84,85; 72.9% of strains were thus categorized. When the nontypable group was added to this, 90.7% of all strains were accounted for. The remaining types included 10 different patterns. Four of these were of interest since they were part of the classic group I category, which 20 years ago was the all-encompassing epidemic pattern typified by type 52,52A,80,81. This group has not disappeared; but neither has it resumed an epidemic behavior. Type 84,85 was predominant to an extent not seen in previous years. The three associated patterns included type 84, type 85 and type 47,53,54,75,83A. It was from this latter pattern that the predominant types 84 and 84,85 emerged in 1971. There remain three types of interest: 94, 95 and 94,96. These are relatively new types on the world scene, and it had been anticipated that once encountered they might tend to increase in frequency. This has not been the case; they remain essentially rare entities, readily recognized but without the pervasive capability which could make them epidemic ologically important.

The diversity of types in 1977-1978 was certainly diminished in contrast to the picture seen in 1976-1977. Then there were 23 types recognized. In 1977-1978, only 11 types were differentiated.

Table 1. Bacteriophage Types of *Staphylococcus aureus*
1977-1978, ISR

Phage Type	No. of Strains	% of Total
52, 52A, 79, 80, 81	1	0.2
29, 52, 79	1	0.2
52, 80	1	0.2
81	5	1.4
47, 53, 54, 75, 83A	1	0.2
84	5	1.4
84, 85	253	72.9
85	3	0.8
94, 96	7	2.0
95	3	0.8
96	5	1.4
Nontypable	62	17.8
Total typed	347	
No. of different types	11	

Comparison of the numerically predominant types since 1971 is shown in Table 2. It is clear that the burn ward patient population was subjected to colonization primarily from the two types, 84 and 84, 85. A gradual increase in incidence of type 84, 85, which was first encountered in 1973, culminated in its marked predominance in 1975, when type 84 was only the third most common type. The succeeding two years showed a reappearance of type 84 up to 23.9% of isolates in 1977, when a unique rise in incidence of nontypable strains occurred. But in 1978, when a striking increase in staphylococcemia was seen, the type 84, 85 strains predominated in a manner comparable only to the behavior of type 84 in 1971-1972. Type 84 was negligible in incidence, and the 94, 96 combinations were equally insignificant.

Table 2. Predominant Bacteriophage Types of
Staphylococcus aureus, 1971-1978, ISR

Phage Type	Year and % of all Strains							
	1971	1972	1973	1974	1975	1976	1977	1978
84	74.6	72.5	61.1	55.6	3.8	20.0	23.9	1.4
84, 85	0	0	5.2	19.2	61.3	49.7	35.3	72.9
94	0	2.1	3.5	2.3	9.5	0.8	0	0
94, 96	0	0	0	0	0	0.1	0.2	2.0
96	0	0	0	0	0	1.6	8.7	1.4
Nontypable	21.6	19.9	20.5	15.6	16.9	17.7	32.3	17.8

SEPTICEMIA DUE TO STAPHYLOCOCCUS AUREUS, 1977-1978

In any circumstance in which a species of bacteria is present in epidemic proportion, the question of existence of fundamental differences in virulence among strains arises. In the situation presented by the prolonged epidemic of *S. aureus* in the ISR burn wards, it is a matter of concern to differentiate invasive from non-invasive strains, if such differences exist. One criterion for such a procedure is scrutiny of the identities of septicemic strains, in contrast to the remainder of the population, which colonizes wounds and respiratory and urinary tracts.

The phage types of blood stream isolates have been studied on an annual basis for the past 10 years. A comparison for the period 1975-1978 is shown in Table 3. It has been evident that the great predominance of blood stream strains has corresponded to the predominant type of the whole staphylococcal flora. However, in 1975, five different types were collected, and in 1976-1977, four different types. In 1977-1978, however, only the predominant type, 84,85, was recovered from blood. All of the blood stream strains were not typed, and it is of course possible that further typing would have uncovered additional types. However, 68 strains over a seven month period represented a not inconsiderable increment. With a situation in which more staphylococci were being found in blood cultures than had previously been the case, it is highly probable that the epidemic strain has simply overshadowed other phage types, and has become the only blood stream strain to be found

Table 3. Phage Types of *Staphylococcus aureus* from Blood Cultures on Burned Patients

Phage Type	No. Patients with Strains Typed and No. of Strains per Type: 1975-1978					
	1975		1976-1977		1977-1978	
	Patients	Strains	Patients	Strains	Patients	Strains
84, 85	36	110	29	65	28	60
84	1	2	13	30	0	
83A, 85	2	2	0		0	
94, 96	0		1	1	0	
94	2	13	0		0	
29, 52	2	2	0		0	
52, 52A, 80, 81	0		3	3	0	
29, 79, 84	0		1	1	0	
Nontypable	4	7	6	8	4	8
No. Patients with Staphylococcemia with strains typed	44		40		29	

ANTIBIOTIC SENSITIVITY OF BLOOD STREAM STRAINS OF STAPHYLOCOCCUS AUREUS, 1977-1978

The sensitivity of S. aureus to antibiotics has frequently been expressed as a proportion of the number of strains tested, but the chronologic progression of events that make up these totals are not disclosed by a simple total. Shifts from sensitive to resistant and vice versa have been detected for several antibiotics. The dynamics of this process were described for circumscribed intervals in an earlier report¹, but a more detailed scrutiny was made for the period being described. In the following tables, the changes in sensitivity levels over the 1977-1978 reporting period are set down in monthly increments. The resulting patterns cast new light on the capacity of S. aureus strains of a single phage type epidemic to change in sensitivity abruptly and extensively. The phenomenon was especially noteworthy in the behavior of staphylococci toward the methicillin group of antibiotics.

Inhibition at an antibiotic level of 6.25 ug/ml or less has long been regarded as representing a sensitivity of a bacterial strain, and this criterion was applied in this and in earlier studies. The methicillin group of semi-synthetic penicillins, which included methicillin, oxacillin and nafcillin, were tested by monthly increments as shown in Table 4. In this manner, changes in sensitivity, or the emergence of resistance, could be placed in a meaningful time frame. The rate at which changes occurred, and their magnitude, was far greater than had been anticipated. In October 1977, all strains tested (39) were methicillin-resistant. Then, abruptly, sensitive strains appeared; in November, 27% were inhibited by 6.25 ug/ml; in December, 17%, and then, in January, all strains recovered were sensitive. This sensitivity prevailed through February, and then in March, 30% of the strains were resistant. Then, with equal rapidity, resistance reappeared. In April and May, 34 out of 35 strains were resistant to methicillin. In June and July, the division between sensitive and resistant was less clear-cut; one-third of the isolates were resistant. In August, all but one of the blood stream isolates were sensitive. In September, at the end of the observation period, 87% of the strains recovered from blood were resistant. This re-established a population similar to that seen a year earlier.

Although there is a widespread impression that methicillin-sensitivity reflects or parallels that of other semi-synthetic penicillin analogues, observations on oxacillin and nafcillin have not confirmed this concept. For oxacillin, shown in parallel with methicillin in Table 4, strains collected in October were 91% resistant, while in November, 96% were sensitive. This sensitivity persisted through December. Then, in January and February, 83% of the strains shifted to resistance. In April, 22 out of 27 strains were sensitive to oxacillin, and the species remained essentially sensitive for the remaining five months.

Nafcillin was virtually ineffective against S. aureus for the entire

1. Lindberg RB, Latta RL, Pruitt BA, Jr, Mason AD, Jr: Emergence of methicillin-resistant Staphylococcus aureus type 84 and 84,85 in burned military personnel. USA Inst Surg Res Ann Rpt FY 76, BAMC, Ft Sam Houston, Tx. p. 81.

Table 4. Methicillin, Oxacillin and Nafcillin: MIC vs S. aureus: Oct 1977-Sep 1978

Conc. ug/ml	Month and No. of Strains Inhibited at Each Level											
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
METHICILLIN												
>25	2	2	1				13	13	2			7
25	35	11	11									4
12.5	2	34	30			7	8		3	4	1	19
6.25		17	9	1	3	12	1		5	7	5	3
3.1		1		1	3	3			2	3	3	1
1.5				5	4	1						
<0.78				3	10							
OXACILLIN												
>25	34	1		8	13	1	2					
25	1				2		2		1			
12.5	4	1	3		1		1		1	1		1
6.25	2	28	4	1	3	4	11		8	3	2	7
3.1	2	10	35		1	15	11		3	2	1	11
1.5			3						6	8	4	13
<0.78		13				1			1	3	1	1
NAFCILLIN												
>25	30	25	5	9	16	23	18	5	10	7	4	26
25	12	26	9		4		4	3	2	3	1	9
12.5		3					5	2		1		
6.25												
3.1												
1.5												
<0.78												

observation period. Only two strains out of 253 were inhibited by 6.25 ug/ml, while 178 were resistant to 25 ug/ml. Thus, the three methicillin and oxacillin were actually, at some times, reciprocal in their sensitivity, as shown thus:

	Months and Reaction											
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Methicillin	R	R	R	S	S	S	R	R	S/R	S	S	R
Oxacillin	R	S	S	R	R	S	S	S	S	S	S	S

R: Resistant S: Sensitive

The phenomenon of "methicillin-resistant staphylococci" has been described in terms that imply that it is an absolute phenomenon, generated by exposure to a given antibiotic and persisting for long periods. The observations set down above indicate that it can, instead, be a transitory, frequently reversed phenomenon. Since methicillin was virtually never used during the reporting period, the possibility arises that exposure to antibiotic in patients is not necessary to have resistance appear.

In view of the behavior of staphylococci toward methicillin and its analogues, a comparable chronologic comparison was carried out with other major groups of antibiotics. Table 5 summarizes the behavior to the aminoglycosides, gentamicin and tobramycin. These are the same staphylococcus strains which contributed the methicillin data. There is no evidence of linkage of the methicillin and aminoglycosides resistances factors. Sensitivity to aminoglycosides did not vary during monthly intervals. Only 7% of the strains on an annual basis were sensitive (i.e., inhibited by 6.25 ug/ml or less) to gentamicin, and 8.8% to tobramycin. No fluctuations of significance occurred on a monthly basis.

Tetracyclines, including minocin and vibramycin, are part of the test battery of antibiotics routinely used in this laboratory. Sensitivity to these antibiotics is shown in Table 6. The two antibiotics were almost identical in their sensitivity patterns. 94.5% of the strains were sensitive to minocin, and 89.3% to vibramycin. Slightly different resistant patterns were detected. Strains were resistant to vibramycin but sensitive to minocin in January, and in September, the mean sensitivity level for minocin was 2.4 ug/ml, while for tobramycin, it was 5.0. But these fluctuations do not merit assessing the two antibiotics as distinctive in their action or response. There was no monthly fluctuation or reversal of antibiotic sensitivity in a manner like that observed in the case of the methicillins, and no indication that there was any linkage with the factors responsible for their labile variation in sensitivity to the methicillins.

Three antibiotics remain in the battery of 10 selected for routine testing of gram-positive organisms. The behavior of these strains toward keflin, vancomycin and clindamycin are shown in Table 7.

Keflin was highly active against these staphylococci throughout the 12

Table 5. Aminoglycosides: Gentamicin and Tobramycin vs. S. aureus: Oct 1977-Sep 1978

Conc. ug/ml	Oct	Nov	Dec	Month and No. of Strains Inhibited at Each Level											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep						
<u>GENTAMICIN</u>															
>25	30	61	51	8	17	20	26	9	10	14	4	34			
25	5	1			1										
12.5	3	4			2										

6.25	1	2										1			
3.1					1	1									
1.5	1	2	1		3	3						1			
<0.78			1		2			2							

<u>TOBRAMYCIN</u>															
>25	35	59	57	8	11	20	22	10	11	15	8	34			
25	1														
12.5					1										

6.25	3	6				1						1			
3.1		1				1									
1.5		2			1	1		2							
<0.78	1		2	1	5	1		1							

Table 6. Tetracyclines: Minocin and Vibramycin vs. *S. aureus*: Oct 1977-Sep 1978

Conc. ug/ml	Month and No. of Strains Inhibited at Each Level											
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
MINOCIN												
>25												
25		2			1		3					
12.5		5	1	1	1	1	2			1		
6.25	12	37	19	1		6	4	1	1		1	2
3.1	25	21	28	2	6	6	7	3		6	5	15
1.5	4	3		4	4	6	6	1	3	6	1	15
<0.78	2	4	4		8	6		9	7	3	1	2
VIBRAMYCIN												
>25						1						
25							1					
12.5	4	14	3	7		3	1			1		
6.25	25	41	44	1	5	8	11	3	3	6	4	22
3.1	20	2	5	1	2	6	7	6	5	7	4	11
1.5		1			3	1			3	1		
<0.78	4	3			7	6		5			1	

Table 7. Keflin, Vancomycin and Clindamycin: MIC vs S. aureus: Oct 1977-Sep 1978

[illegible]

month observation period. Only 11 out of 316 strains (3.4%) were resistant and such strains were scattered over the whole year.

Vancomycin, which has been the principal drug used for controlling staphylococcal septicemia in recent years, was effective against virtually all strains; only one resistant isolate was found. There was no significant fluctuation over the months recorded.

Clindamycin exhibited a striking disappearance of effectiveness. Only in October 1975 were strains sensitive to this drug recovered. After that time, no more sensitive strains were recovered. In 1975, 98% of strains were sensitive to clindamycin; in 1976-1977, 75% were sensitive. In 1977-1978 11.8% were sensitive, and after October, none. With these three antibiotics, no fluctuation in sensitivity was seen. There was no evidence that linkage between methicillin-resistance and resistance to other antibiotics occurred in this population of staphylococci. The loss of sensitivity to clindamycin was abrupt, and no sign of reversal occurred during 1977-1978.

It would appear that sensitivity to methicillin type antibiotics is a highly labile entity. There was no parallel fluctuation in sensitivity to other antibiotics

PRESENTATIONS

Lindberg RB: Burn wound infection and sepsis. presented at ASM annual meeting in Atlanta, Georgia, 4-11 June 1978.

PUBLICATIONS - None

ANNUAL PROGRESS REPORT

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Previously developed phage typing system for *Serratia marcescens* was re-examined. Immunity to lysis developed among three propagating strains. New phages were recovered to remedy this defect.

Four phages of the earlier set have been maintained in adequately high titer. Twelve new phages were recovered from raw sewage, and cross-reaction patterns of identity for 16 phages have been completed. Thirteen phages were selected to comprise the typing set. This improved typing system made possible precise differentiation of *Serratia marcescens* strains.

A total of 287 strains of *Serratia marcescens* were typed with the new 13 phage set. Each of the typed strains was included in an antimicrobial sensitivity study utilizing a modification of the Kirby-Bauer plate technic.

Burns
Serratia
Bacteriophage
Sensitivities
Humans

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TYPES OF *SERRATIA MARCESCENS* FROM BURN WOUNDS OF
MILITARY PERSONNEL

In 1968 a seven-phage typing system was developed and used to differentiate *Serratia marcescens* strains isolated from burn patients at the Institute of Surgical Research. This system provided an accurate, sensitive technic for the precise identification of *Serratia* strains in a burn patient population highly susceptible to opportunistic infection. Stock bacteriophage had been prepared in large amounts to insure a constant titer and the purity of each phage. When these stock solutions of bacteriophage were depleted, attempts to propagate three phage revealed the rapid emergence by the host organism with subsequent total immunity to lysis. Four *Serratia* phage propagating strains remained susceptible to infection and lysis. These four phages did not make up an adequate type differentiating set. It was evident that if it was to be usable, the typing set would require addition of new phages with appropriate spectra of activity. This procedure was implemented.

MATERIALS AND METHODS

Sewage effluent from different levels of treatment were collected. Twelve bacteriophage suspensions were obtained and purified with titer reaching 10^8 to 10^{10} plaque forming units per ml. The technics and methods used to obtain these phage suspensions were those previously used in this laboratory and described in detail at that time (1). The 12 new phage isolates and the earlier phages 1, 9, 11 and 18 of the original set were used to establish a cross-reaction pattern by determining the lytic reactions of the 16 phage suspensions against all propagating strains (Table 1). Thirty-eight other *Serratia* strains, picked at random, yielded 13 distinct phage strains (Table 2). Type strains were selected from among the collection and the routine test dilution (RTD) was determined using a modification of the method described by Blair and Williams (2). Two hundred eighty-seven *Serratia* isolates were phage typed utilizing the 13 phage-component set. Phage typing was carried out according to the method previously described by Lindberg and Latta (3) for phage typing of *Pseudomonas aeruginosa*.

RESULTS

The typing system, as reconstructed, consisted of 13 phages of separate identity. The new typing set permitted precise strain identification. Table 2

1. English VE, Latta RL, Brame RE, Lindberg RB: Development of a bacteriophage system for organisms of the genus *Serratia*. USA Inst Surg Res Ann Rpt FY 68, BAMC, Fort Sam Houston, Texas 78234. Section 32

2. Blair JE, Williams REO: Phage typing of staphylococci. Bull. WHO 24: 771-784, 1961.

3. Lindberg RB, Latta RL: Phage typing of *Pseudomonas aeruginosa*: Clinical and epidemiologic consideration. J. Infec Dis 130: 533-542, 1974.

Table 1. Cross-reaction Pattern - Phage vs. Phage Host

Host:	Phage:															
	1	9	11	18	20	22	24	26	28	30	31*	32	33*	34	35*	36
1	4										1				4	3
9		4	3	1	2	3	3	3	3	4	4			4	4	4
11		1	4	2				4		4	4			4	4	4
18		3	3	4	4	4	4	3	2	4	1			4	4	4
20			1		4											
22	3		4		2	4	4	4		4	4			4	4	4
24	1		2		1	3	4	3		4	4			3	4	4
26					1		4	4	1	3	4			4	4	4
28								3	4					1	3	3
30			2				3	2		4	4			4	4	4
31	3		3		2	3	3	2		3	4			3	3	3
32												4	4			
33												3	4			
34			2				3	3		4		4	4	4	4	4
35							1	4	2	1	3			3	4	3
36		1		1					2						4	4

○ Reaction of phage with propagating host.

INTERPRETATION OF LYTIC REACTION:

- 4 Complete lysis of host strain by bacteriophage
- 3 Almost, but not confluent lytic reaction
- 2 Semi-confluent lysis
- 1 50-100 plaques

* These phage suspensions deleted from the set since they duplicated other phage reactions.

shows the lytic activity for each of the 13 phages, with a range of activity from 3.5% to 83.6%. Thirteen phages characterized by this type of variation could produce almost infinite combinations of reaction patterns.

Table 2. Lytic Activity of Individual Phage Strains *

Phage Number	Number of Organisms Susceptible to Lysis	Percent of Lytic Activity of Phage
1	136	47.4
9	47	16.4
11	176	61.3
18	84	29.3
20	22	7.7
22	185	64.5
24	161	56.1
26	240	83.6
28	68	23.7
30	170	59.2
32	10	3.5
34	240	83.6
36	228	79.4

* Percentage based on 287 organisms typed

Two hundred eight-seven *S. marcescens* strains collected over a 5-year period were typed with the phage set, and 101 individual phage patterns were recognized. The individual phage patterns could be long and cumbersome to work with in their complete form. Hence, each type pattern was assigned an identifying code number. The number of strains per type averaged 9.3. From 1974-1976, four predominant phage type patterns averaged only 11 strains per pattern. In 1977 and 1978 no predominance of phage type occurred.

Table 3 summarizes the incidence of *Serratia* types as they occurred in burn ward patients. Eighty *Serratia* strains were isolated from April through December of 1974, and 30 different phage types were differentiated. Twenty-five (31.2%) of the 80 strains were of one of the two predominant phage types. From a total of 21 patients positive for *Serratia*, seven (33%) had one of the two predominant types of colonizing strains. The remaining 28 types recovered were distributed among 14 patients and occurred as well among those patients who harbored the predominant type.

In 1975, 158 *Serratia* strains were typed. These were distributed among 41 phage types, with a total of 47 patients positive for *Serratia*. Only one type, A-13, showed a degree of predominance, with 75 strains (47%) of the total strains collected. Twenty-one (44.7% of 47 patients positive for

Table 3. Phage Types of *Serratia marcescens* in Burn Patients -
Institute of Surgical Research, 1974-1978

Year	Total		No. of Positive Patients	Predominant Type	Patients-This Type		Strains-This Type	
	Strains	Type			Number	Percent	Number	Percent
1974 (Apr-Dec)	80	21	21	B-6 C-	3 4	14.3 19.0	17 8	21.2 10.0
Total both predominant types:								
1975	158	41	47	A-13	21	44.7	75	47.0
1976	35	18	23	H-1	5	21.7	6	17.1
1977	9	9	8	None				
1978 (Jan-Jun)	5	3	2	None				

Serratia harbored the predominant type.

In 1976, 35 *Serratia* strains were isolated from the burn patient population, and 18 phage types were identified. Type H-1 was the predominant type; it made up 17% of the strains typed. Five out of 23 patients positive for *Serratia* harbored type H-1.

During 1977 and through June 1978, *Serratia* strains were extremely rare in the burn ward. Only nine strains were recovered in 1977, seven patients had one strain each, and one patient had 2 strains of different types. In 1978, four isolates of *Serratia* were recovered, with three patterns found in two patients.

A more detailed study of the strain that evidently caused an epidemic type of outbreak in 1975 is shown in Table 4. This outbreak was characteristic of similar *Serratia* epidemic episodes which have occurred in the past at this Institute. A strain of type A-13 was isolated from a urine specimen on 2-17-75. On 7-15-79 another strain was recovered from bronchial secretions. Two patients in the month of August were positive for *Serratia* of type A-13 recovered from several of their urine and Luken's specimens. Throughout the remainder of the year, repeated Luken's tube, blood, urine, biopsy and postmortem specimens of 17 other patients were shown to have harbored phage type A-13. Its final recovery came from a Luken's tube specimen obtained on 1-1-76 from a patient who had been admitted in 1975 during the height of the outbreak.

There was a distinct increase of blood cultures positive for *Serratia* during the years 1974-1976 (Table 5). Seven patients in 1974, 10 patients in 1975 and 6 patients in 1976 had one or more bacteremic or septicemic episodes with *S. marcescens*. Prior to those years, there had never been more than one patient in a given year with positive *Serratia* blood cultures. The explosive increase of *Serratia* positive blood cultures did not appear to be correlated with any given phage type.

DISCUSSION

The determination of strain identity and distribution of *S. marcescens* by phage typing offered a means for following the epidemic pattern of this organism in burn patients. Small epidemic outbreaks identified by the expanded set were similar in character to those identified by the seven phage set used at ISR from 1968 to 1974. The 13 phage set offered a more precise strain differentiation, since it was capable of differentiating closely related strains. Use of this more sophisticated system results in more different types being recognized, so that the number of strains in a predominant type is fewer than was the case with a smaller number of phages.

S. marcescens recovered from the burn patients at this Institute during this 10 year bacteriophage typing study appeared most often on the burn ward as a transient inhabitant. A few small epidemic episodes did occur, probably as a result of patient-to-patient transmission and subsequent colonization of a

Table 4. Survey of Predominant Type A 13

PATIENT	DATE OF CULTURE	SOURCE	PATIENT	DATE OF CULTURE	SOURCE
1	2-17-75	Urine	9	9-15-75 (Post mortem)	Spleen RLL
2	7-15-75	Lukens	10	9-19-75	Blood
3	8-4-75	Urine	11	9-29-75 (Post mortem)	RUL Blood Liver Spleen RUL RLL LUL LLL Tissue # 1
4	8-21-75	Lukens	12	10-7-75 10-8-75 10-10-75 10-10-75 10-16-75 (Post mortem)	Lukens Lukens Urine Blood PM Blood Spleen RUL LUL LLL
5	9-1-75	Bx. Rt. Hip	13	10-15-75	Urine
	9-15-75	Blood	14	10-21-75 10-22-75 10-23-75 10-23-75 11-14-1 12-2-75	Blood Blood Lukens Blood Lukens Tracheal Aspirate
	9-17-75 (Post-mortem)	Liver Tissue # 4	15	10-22-75 10-22-75 10-24-75 10-24-75 10-29-75 (Post mortem)	Surface swab Lukens I.V. tip Urine LLL PM Blood RLL
6	9-5-75 9-10-75 9-21-75 10-1-75 10-9-75 10-15-75 10-17-75 10-20-75 10-24-75 10-30-75 (Post mortem)	Lukens Lukens Lukens Lukens Blood Lukens Lukens Wrist Urine Tissue # 1	16	11-2-75 11-3-75	Urine Urine
7	9-8-75 9-11-75 (Post mortem)	Lukens Spleen LLL	17	11-8-75	Blood
8	9-8-75 (Post mortem)	Liver Spleen LLL LUL RLL RUL Tissue # 4	18	11-10-75 (Post mortem)	Tissue # 4
			19	11-28-75	Lukens
			20	12-1-75	Throat
			21	1-2-76	Lukens

Table 5. Patients with Serratia Positive Blood Cultures

Year	Number of Patients	Number of Isolates
1968	0	0
1969	1	1
1970	0	0
1971	1	4
1972	0	0
1973	1	1
1974	7	14
1975	10	20
1976	6	7
1977	1	1
1978	0	0

predominant type on the ward.

The diminished incidence of Serratia in 1977 and 1978 reflected a period when the organism was a transient and insignificant portion of the burn wound flora. Such quiescent periods have been characteristic of this species, but they have usually been followed by periods of exacerbation when epidemic outbreaks of the organism were seen.

Bacteriophage typing of Serratia constitutes a reliable system for continued scrutiny of the epidemiology of S. marcescens recovered from the burn patient.

PRESENTATIONS/PUBLICATIONS - None

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- SENSITIVITY TO
SULFAMYLON OF PSEUDOMONAS AERUGINOSA RECOVERED
FROM BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

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A representative sample of 141 strains of *Pseudomonas aeruginosa* were assessed for sensitivity to Sulfamylon^R using an agar dilution technic. The strains were highly sensitive; in comparison with previous years, no increase in resistant strains was detectable. The median sensitivity was 0.089% in comparison with 0.117% in 1977. Epidemic episodes could be shown to be associated with strains at the upper limit of sensitivity. Sulfadiazine resistance was high; over 80% of isolates were sulfadiazine resistant.

Pseudomonas
Sulfamylon
Burns
Silver sulfadiazine
Topical therapy
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF
TROOPS WITH THERMAL INJURY -- SENSITIVITY TO SULFAMYLON
OF PSEUDOMONAS AERUGINOSA RECOVERED FROM BURNED SOLDIERS

Topical therapy has become an established component of burn wound treatment, and control of invasive infection and of subsequent sepsis relies heavily on this modality. The principal agents presently used for topical treatment are Sulfamylon[®] and silver-sulfadiazine, and both of these are being used in this Institute. At the time of this report, each of these materials was being used alternately on a 12-hour basis. The rationale for this approach was, in part, the control of emergence of drug resistant forms of bacteria. Whether resistant forms play an important role in the development of burn wound sepsis is, at present, conjectural. Objective data on resistance to these two important therapeutic agents is of fundamental importance in maintaining a rational approach to control of burn wound infection. The entire experience with antimicrobial agents, since the introduction of sulfamilamide in the 1930's and of penicillin in the 1940's, has been one of continuous appearance of resistant strains of bacteria, to negate the initial successes which such agents conferred. A classic exception to this rule is the behavior of the beta-hemolytic group A streptococci toward penicillin: This species has remained as susceptible to penicillin as it was in 1940. Another such exception has been demonstrated in this laboratory in the past decade: Pseudomonas aeruginosa sensitivity to Sulfamylon has not altered essentially since the drug was introduced for burn wound treatment in 1965. The behavior of Pseudomonas isolates from burns has been monitored, using an agar dilution plate technic developed here, to include the entire spectrum of colonized and infected sites, and annual summaries of sensitivity have been compared to detect any significant changes in in vitro susceptibility.

SENSITIVITY OF PSEUDOMONAS AERUGINOSA TO SULFAMYLON

This report covers the period from 1 October 1977 through 30 September 1978. One hundred forty-one strains were tested for Sulfamylon susceptibility. The concentrations used for testing ranged from 2.5%, by equal volume dilution, to 0.019%. A presumptive level of 0.312% has been established, by clinical experience and laboratory model studies, as the upper limit for designation of a strain as sensitive. Since the drug is applied in a 11.1% suspension in a cream base, even strains requiring 0.625% or 1.25% for suppression are in the presence of an effective concentration, although they may be regarded as in some degree resistant. The 0.312% concentration is 1/32 of the actual concentration of Sulfamylon. In 1978, 76.5% of strains tested were inhibited by 0.312% of Sulfamylon.

The range of sensitivity is shown for 1977-1978 in Table 1. There was a shift upward in the inhibitory levels, compared to the previous year's results. 23.5% of the strains required more than 0.312% of Sulfamylon for inhibition; in 1977-1978, the proportion was 17.4% of the strains tested. The difference is not beyond the range of fluctuations that have been seen in recent years.

Table 1. Sensitivity to Sulfamylon for *P. aeruginosa*
1977-1978

No. Strains	Concentration Required for Inhibition ug/%	% of Total Tested
16	1.25	11.3
17	0.625	12.2
16	0.312	11.3
26	0.156	18.4
48	0.078	34.0
12	0.039	8.5
5	0.019	3.5
1	<0.019	0.8
141		

This fluctuation in sensitivity to Sulfamylon is fundamental to an understanding of the *in vitro* response of *P. aeruginosa* to the drug. Sensitivity levels in preceding years are shown in Table 2, for the years 1970-1978. There was a marked shift toward a higher level of resistance in 1972, but the phenomenon was transitory, and in the next three years resistance shifted back toward a median level that has been very consistent. The largest increment of strains, in terms of a given inhibitory level in 1977-1978, was the group inhibited by 0.078 gm/% or less. This range approaches the lower level of measurable resistance.

The changes that have occurred in sensitivity are most clearly visualized. This information is shown in Table 3. Comparison of annual levels makes it clear that a moderate increase in resistance of *P. aeruginosa* occurred in 1972; after that time, there was an increase in resistance, but no progressive rise. Instead, it leveled off at a point higher than was observed in the first four years of its use, and has stayed substantially unchanged since 1973.

A median value, at which 50% of strains are inhibited, offers still another perspective on the constancy of the susceptibility of *P. aeruginosa* to Sulfamylon. These values are shown in Table 4. In three years: 1970, 1974 and 1978, the median inhibitory concentration was less than 0.100%. It was evident that the ubiquitous pseudomonads are not increasing their resistance to Sulfamylon.

In recent years, with the extensive use of silver sulfadiazine as a topical agent in burns, concern has arisen over the increase in sulfadiazine resistance that has been emerging. In view of this concern, the strains assessed for Sulfamylon sensitivity were simultaneously tested for sulfadiazine resistance using a similar agar plate dilution technic. The results of these

Table 2. Inhibiting Concentrations of Sulfamylon for *P. aeruginosa*
1970-1978

Year & % of Total	No. of Strains	Concentration of Sulfamylon in gm/% and No. of Strains Inhibited									
		1.25	0.625	0.312	0.156	0.078	0.039	0.019	≤0.019		
1970	296	0	0	65	83	83	59	6	0		
				21.9	28.0	28.0	19.9	2.0			
1971	286	0	48	41	56	57	65	13	0		
			16.7	14.3	19.5	19.3	22.7	4.5			
1972	463	29	212	46	88	31	37	15	5		
		6.3	45.8	9.9	19.1	6.7	7.9	3.2	1.1		
1973	285	4	14	85	85	52	32	12	1		
		1.4	4.9	19.8	29.8	18.3	11.2	4.2	0.4		
1974	437	5	59	78	97	97	86	11	4		
		1.1	13.5	18.0	22.2	22.2	19.7	2.5	0.9		
1975	637	13	113	108	155	68	147	28	4		
		2.0	17.7	16.9	24.3	10.7	23.1	4.5	0.64		
1976-77	698	4	118	135	295	95	18	23	10		
		0.57	16.91	19.37	42.2	13.6	2.5	3.3	1.4		
1977-78	141	16	17	16	26	48	12	5	1		
		11.3	12.0	11.3	18.4	34.0	8.5	3.5	0.7		
TOTAL	3243	71	581	574	885	531	456	113	25		
		2.1	17.9	17.6	27.2	16.3	14.0	3.4	0.7		

Table 3. Cumulative Sensitivity to Sulfamylon of P. aeruginosa
1968-1978

Year	No. of Strains	Percent of Strains Inhibited at Sulfamylon gm/%							
		1.25	0.625	0.312	0.156	0.078	0.039	0.019	0.019
1968	294	100	100	95.1	60.4	45.8	14.1	1.7	-
1969	385	100	100	96.5	50.0	26.9	7.7	0.5	-
1970	296	100	100	100	78.0	49.9	21.9	2.0	-
1971	280	100	100	82.9	68.3	48.3	27.9	4.7	-
1972	463	100	93.7	48.0	38.0	19.0	12.3	4.3	1.1
1973	285	100	98.1	81.3	57.0	33.5	16.1	3.2	0.4
1974	437	100	99.0	85.5	67.5	45.3	23.1	2.4	0.9
1975	637	99.8	97.8	80.1	63.2	38.9	24.2	5.0	0.6
1976-77	698	100	99.4	82.5	63.2	21.0	7.3	4.7	1.4
1978	141	100	98.1	83.5	64.3	34.3	17.9	4.5	0.9

Table 4. Median Value of P. aeruginosa Sensitivity to Sulfamylon
1968-1978

Year	No. of Strains	Median Inhibitory Level % Concentration
1968	294	0.136
1969	385	0.176
1970	296	0.068
1971	280	0.125
1972	463	0.316
1973	285	0.111
1974	437	0.086
1975	637	0.125
1976-77	698	0.117
1978	141	0.089

tests are summarized in Table 5.

Table 5. Sensitivity to Sodium-Sulfadiazine of P. aeruginosa
1978

No. of Strains	Inhibiting Concentration in %	% of All Strains Tested
95	> 1.25	67.4
0	1.25	-
0	0.625	-
1	0.312	0.8
2	0.156	1.4
4	0.078	2.8
9	0.039	6.4
15	0.019	10.5
4	0.0095	2.8
7	0.0047	5.1
0	0.00235	-
4	0.002375	2.8

When growth inhibition, or MIC technic was used, two groups of strains were delineated. 67.4% of strains were totally sulfadiazine resistant, and grew in the presence of 1.25% sulfadiazine. An additional 11.4% of the strains required 39 ug% or more for inhibition; these strains would be regarded

as resistant to a significant degree. Thus, 78.8% of the strains were essentially sulfadiazine resistant. If this population were tested with the disc-diffusion technic, the designation of resistant would probably be higher.

The implications of the appearance of a large population of sulfadiazine resistant P. aeruginosa strains in a burn population in which silver-sulfadiazine is used extensively are potentially grave, the future effectiveness of silver-sulfadiazine is impugned by this development.

Continued monitoring of opportunistic burn wound pathogens for sensitivity to topical agents is a major part of a program directed toward continued control of burn wound infection and invasion. The behavior of two major topical agents in this in vitro study constitutes a guideline for future therapy.

PRESENTATIONS AND/OR PUBLICATIONS - None

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The search for unusual oxidative and fermentative gram-negative bacilli has been continued. This population includes the most probable source of new opportunistic invaders, in view of past experience on nosocomial infections in burns, 10 species, including 4 *Pseudomonas* species, were recognized in clinical specimens (primarily wound and sputum cultures). Five species appeared in episodes of bacteremia. The incidence of unusual opportunistic invaders fluctuates over a long term, but such differentiation will aid in detection of new infection problems which will inevitably emerge on the basis of the history of burn wound infection.

Burns
Oxidative microorganisms
Pseudomonas
Acinetobacter

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF
TROOPS WITH THERMAL INJURY -- NON-FERMENTATIVE
GRAM-NEGATIVE BACILLI IN BURNED SOLDIERS:
NEW POTENTIAL OPPORTUNISTIC PATHOGENS

Since opportunism characterizes gram-negative bacterial infection in burn wounds, it is highly desirable that cultures from burned patients be scrutinized with especial care to detect unusual oxidative or fermentative bacteria which are not usually recognized as potential pathogens for tissue-damaged, immune-deficient hosts. This study has included specific effort directed toward late-appearing species which may emerge only after extended incubation. The experience of encountering virulent epidemic strains of such unusual species as *Providencia stuartii*, in the 1969-1973 period, is a constant reminder that detailed scrutiny of flora of the burned patient is indicated.

UNUSUAL GRAM-NEGATIVE FLORA ON BURN PATIENTS

It was not unexpected that the greater proportion of unusual gram-negative bacilli would be recovered from external sites, i.e., sputum and wound cultures. Table 1 sets forth 10 species, with a total of 56 strains. This is a smaller number of strains than have been collected in recent years, although the number of species was in the same range for several years. The four species of *Pseudomonas*: *cepacia*, *putida*, *fluorescens* and *stutzeri* have been observed as sporadic colonizers or contaminants in previous years.

Table 1. Unusual Gram-Negative Species Recovered
from Clinical Bacteriology Specimens, 1977-1978

Species	Source and Number of Strains		
	Blood	Sputum	Wound
<i>P. cepacia</i>	4	3	1
<i>P. putida</i>	1	2	6
<i>P. fluorescens</i>	0	16	8
<i>P. stutzeri</i>	0	1	0
<i>Acineto. anitratum</i>	2	3	1
<i>Acineto. lwoffii</i>	0	0	1
CDC Gp 5E-2	1	0	0
<i>Aeromonas hydrophila</i>	0	0	1
<i>K. oxytoca</i>	0	1	5
<i>Entero. agglomerans</i>	1	0	0

In addition to clinical cultures, postmortem bacteriology has yielded a small number of bacteria rare in burns. A summary of results observed in the 1977-1978 period is shown in Table 2. Again, a small number of primarily oxidative species was recovered.

Table 2. Unusual Gram-Negative Species Recovered from Autopsy Bacteriologic Specimens, 1977-1978

Species	No. of Patients	Source	No. of Isolates
<i>P. cepacia</i>	2	Lung, wound	2
<i>P. putida</i>	1	Spleen	1
<i>Acineto. calcoaceticus</i> var. <i>lwoffi</i>	2	Lung, spleen	2
<i>Acineto. calcoaceticus</i> var. <i>anitratum</i>	2	Lung, wound	3
<i>Aeromonas hydrophila</i>	2	Wound	2
<i>Flavobacterium</i> spp	1	Spleen	1
<i>Citro. diversus</i>	2	Wound	2
CDC VE-1	1	Wound	1

P. cepacia and *putida* appeared in both sets of data, as did members of the *Acinetobacter* (formerly *Mima*) group. *Aeromonas hydrophila* was recovered in both sets.

The organisms were sparsely distributed. There was no indication of a transmission sequence between patients. In actuality, the collection was the smallest numerically of any that have been extracted.

The strains were dispersed in a random manner. None could be said to have exhibited pervasive tendencies. The infrequently occurring species should be monitored, but there is as yet no indication of rising severity in such infestations.

PRESENTATIONS/PUBLICATIONS - None

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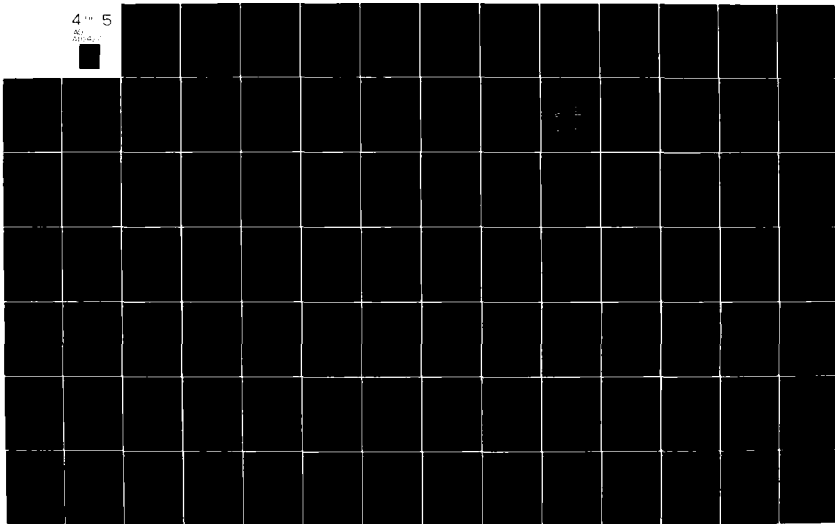
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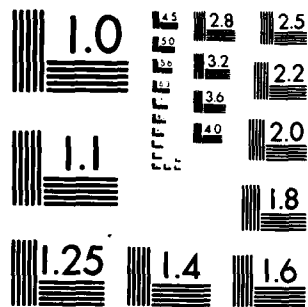
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ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF
TROOPS WITH THERMAL INJURY: CURRENT TRIAL OF ANTISERUM
AGAINST GRAM NEGATIVE BACTERIA

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

Thomas J. Lescher, M.D., Major, MC
Elizabeth J. Ziegler, M.D.
Truman M. Sasaki, M.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

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TROOPS WITH THERMAL INJURY: CURRENT TRIAL OF ANTISERUM
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US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

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Reports Control Symbol MEDDH-288 (R1)

Thermal injury disrupts the normal host response to infection. Burn wound invasion by gram negative bacteria is the major threat to the survival of these thermally injured patients. The purpose of this project is to investigate the effectiveness of an human antiserum in augmenting the patient's defense against gram negative sepsis. The human J5 E. coli 0111 antiserum has been prepared and supplied by Dr. Elizabeth Ziegler of the University of California School of Medicine, San Diego, California.

Pseudomonas
Klebsiella
Wound Infection
Sepsis
Endotoxin
Topical chemotherapy
Humans
Staphylococci

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: CURRENT TRIAL OF ANTISERUM AGAINST GRAM NEGATIVE BACTERIA

Thermal injury disrupts the normal host response to infection. Two significant defects are (1) loss of the skin barrier to bacteria and (2) loss of the normal immunological response to infection. Burns hamper immunologic response by (1) delaying leucocyte migration (2) hindering intracellular lysis of bacteria (3) altering reticuloendothelial phagocytosis and (4) suppressing antibody production. 1 The altered leucocyte migration and depressed immunoglobulin patterns in burn patients have been confirmed at this institute.^{2,3}

Both active and passive immunization have been used to augment the defective immunoglobulin system. Immunization in animal burn models results in striking improvement in mortality, especially against *Pseudomonas* burn wound infection.^{4,5,6,7,8,9} Immunization has been used in burned patients for a number of years in an attempt to prevent burn wound sepsis. The results reported by Sachs, Alexander and Feller were optimistic but remain unconfirmed.^{10,11,12}

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1. Munster AM: Alterations of the host defense mechanism in burns. *Surg Clin North Am* 50:1217-1225, 1970.
 2. Warden GD, Mason AD, Pruitt BA Jr: Suppression of leucocyte chemotaxis in vitro by chemotherapeutic agents used in management of thermal injuries. *Ann Surg* 181:363-369, 1975.
 3. Munster AM, Hoagland HC: Serum immunoglobulin patterns after burns. *Surg Forum* 20:76-77, 1969.
 4. Millican RC, Evans G, Markley K: Susceptibility of burned mice to *Pseudomonas aeruginosa* and protection by vaccination. *Ann Surg* 163:603-610, 1966.
 5. Jones RS: Early protection by vaccines in burns. *Br J Exp Pathol* 52:100-109, 1971.
 6. Jones RJ: Protection against *Pseudomonas aeruginosa* infection by immunization with fractions of culture filtrates of *Ps. aeruginosa*. *Br J Exp Pathol* 49:411, 1968.
 7. Jones RJ: Passive immunity against gram negative bacilli in burns. *Br J Exp Pathol* 51:53, 1970.
 8. Jones RJ, Lilly HA, Lowbury EJJ: Passive protection of mice against *Pseudomonas aeruginosa* by serum from recently vaccinated mice. *Br J Exp Pathol* 52:264, 1971.
 9. Markley K, Smallman E: Protection by vaccination against *Pseudomonas* infection after thermal injury. *J Bacteriol* 96:867, 1968.
 10. Sachs A: Active immunoprophylaxis in burns with a new multivalent vaccine. *Lancet* 2:959, 1970.
 11. Jones CE, Alexander JW, Fisher M: Clinical evaluation of *Pseudomonas* hyperimmune globulin. *J Surg Res* 14:87, 1973.
 12. Feller I, Pierson C: *Pseudomonas* vaccine and hyperimmune plasma for burned patients. *Arch Surg* 97:225, 1968.

or immune serum has been administered.

Data Collection:

The data collection includes 1) primary source of the sepsis, 2) causative organisms, 3) summary of the clinical outcome, 4) predisposing conditions and 5) untoward reactions to the serum. Careful attention is paid before and after therapy to the clinical changes of certain factors such as vital signs, urine output and orientation. CBC, platelet counts and serum BUN, creatinine and glucose are followed each day for three days. However, the evaluation of the worth of this method of administration of sera will depend heavily on the final outcome i.e., survival or death. The accumulated data will be evaluated after 50 patients have been studied to determine if alterations are necessary in the protocol.

Preparation of Antiserum and Dosage:

The antiserum is supplied by Dr. Ziegler et al. The immune serum is collected after immunization with the J5 E. coli 0111 antigens. The serum is given 3 ml/kg intravenously over 30 minutes. Since this is a double blind clinical trial, the participants at this Burn Unit do not know which serum has been given.

Toxicity

The side effects are infrequent but the possibilities include 1) transmission of hepatitis, 2) hemolytic transfusion reaction, 3) febrile reactions associated with pyrogens in the blood or equipment, 4) allergic reaction manifested by urticaria, 5) bacterial contamination of the plasma.

To avoid these problems, the blood is prepared from healthy volunteers who have been screened for communicable disease e.g., hepatitis and syphilis. The blood is collected aseptically, and the serum processed and stored at 4°C. A sample from each donor unit is cultured aerobically and anaerobically. Pyrogenicity is tested by injection of 3 cc of plasma intravenously into each of three rabbits. The type and Rh are recorded so that each patient will receive only appropriate sera. The risk associated with administration of this serum should not be greater than giving fresh frozen plasma. This serum has been given 57 times without evident toxicity.

RESULTS

Since our last report on this protocol, we have had four more patients who have voluntarily participated in this study. We now have

In 1973, Ziegler et al reported on the use of antiserum against J5 mutant *E. coli* 0111 in agranulocytic rabbits infected with different types of gram negative bacteria.¹³ The antiserum was protective against both *E. coli* and *Klebsiella*. The antiserum is prepared by using a mutant *E. coli* which lacks the cell wall side chains.

In nonmutant gram negative bacteria, the "O" side chains are attached to a "rough" cell wall. The "O" side chain gives bacterial cell walls antigenic specificity and differs between the types of gram negative bacteria. The "rough" cell wall, however, is common to these bacteria and appears to be associated with production of endotoxin. Since the J5 mutant *E. coli* 0111 lacks the "O" side chains, antisera made against this mutant theoretically should be effective against all bacteria with a "rough" cell wall. The antisera may protect against infection from many species of gram negative bacteria. This antiserum has been given to 49 patients with proven or suspected endotoxemia with no complications identified but the effectiveness of the antiserum in man has yet to be proven.

PURPOSE

The effectiveness of this antiserum is to be determined by a clinical trial in patients with suspected or proven gram negative endotoxemia. The clinical evaluation of this material has been previously approved by the University of California at San Diego and by the U.S. Army Medical Research and Development Command.

METHOD AND SUBJECTS

Nonimmune human serum and human J5 *E. coli* 0111 antiserum will be compared in a double blind clinical trial. Subjects consist of patients who have proven or suspected endotoxemia from gram negative infections. Patients are entered into the study if one or more of the following findings are present 1) Blood culture which grows a gram negative bacteria, 2) Biopsy which reveals gram negative bacterial burn wound invasion, 3) or a sudden clinical deterioration accompanied by hypothermia, hypotension and glucosuria. The institution of immune therapy will not alter the standard therapy for this problem. Patients are fully informed of both the risks and benefits and a written consent obtained. Results and data will be recorded without knowledge of whether nonimmune

13. Ziegler EJ, Douglas H, Sherman JE, Davis CE, Braude AI: Treatment of *E. coli* and *Klebsiella* bacteria in agranulocytic animals with antiserum to UDP-GAL epimerase-deficient mutant. *J Immunol* 111: 433, 1973.

administered serum in 24 trials with 21 patients. All of these patients except possibly two have had well documented evidence of gram negative sepsis.

We are presently in the process of breaking the double blind code for this protocol and analyzing the data. Results from preliminary trials with human J5 antiserum conducted at the University of California School of Medicine, San Diego, California are encouraging. An evaluation of the prophylactic use of human J5 antiserum in thermally injured soldiers is presently being considered.

PRESENTATIONS

None

FINAL REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

**REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- EVALUATION OF
PSEUDOMONAS AERUGINOSA TOXIN A IN EXPERIMENTAL
RAT BURN WOUND SEPSIS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1977 - 30 September 1978

Investigators:

**Harrel L. Walker, MS
Charles G. McLeod, Jr, Major, VC
S.H. Leppla, PhD*
Arthur D. Mason, Jr, MD**

*** From USAMRIID, Fort Detrick, Maryland.**

Reports Control Symbol MEDDH-288 (R1)

Unclassified

ABSTRACT

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REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
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Reports Control Symbol MEDDH-288(R1)

The search for methods to achieve control of Pseudomonas aeruginosa infection continues with the introduction of aluminum absorbed toxoid developed from Ps. aeruginosa exotoxin. This toxoid induces significant titers of neutralizing and precipitating antibodies for toxin A when given with appropriate adjuvants.

These experiments show that immunization with aluminum phosphate absorbed toxoid failed to protect the septic burn rat model. These and previous experiments show that active immunization with live Ps. aeruginosa provides good strain-specific protection in the same model. No cross protection was demonstrated between strains of Ps. aeruginosa in these experiments.

Pseudomonas
Sepsis
Wound infection

* From USAMRIID, Fort Detrick, Maryland.

STUDIES OF INFECTION AND MICROBIOLOGIC
SURVEILLANCE OF TROOPS WITH THERMAL INJURY --
EVALUATION OF PSEUDOMONAS AERUGINOSA
TOXIN A IN EXPERIMENTAL RAT BURN WOUND SEPSIS

Pseudomonas aeruginosa infection is a recognized common cause of death in burned patients. Though its incidence has been diminished by topical chemotherapy, this infection is not completely prevented by such treatment and may still occur.

Many virulent strains of Ps. aeruginosa produce a potent protein exotoxin which is lethal in a number of animal models as well as to cultured mammalian cells (1). The exotoxin has been purified to homogeneity (3), and apparently acts within cells by inactivating the elongation factor (EF-2) in the same fashion as diphtheria toxin. Iglewski (2) has suggested that exotoxin plays an important role in the pathogenesis of tissue destruction and death related to Pseudomonas infection in a mouse burn model. The evidence that supports this hypothesis includes demonstrations that (a) levels of elongation factor II (EF-2) were decreased in burned infected mice prior to death; (b) passive immunization with antiserum specific for exotoxin (Antitoxin) increased the mean time to death of burned infected mice and also prevented the decrease in EF-2 levels, and (c) nonexotoxin producing strains of Ps. aeruginosa were less virulent for burned mice than strains producing exotoxin. It is suspected that exotoxin is not the sole cause of death in burned infected mice, but that it damages host defense systems and permits the occurrence of overwhelming bacteremia, which is the immediate cause of death.

An aluminum phosphate absorbed toxoid has been developed from exotoxin A by S.H. Leppla (unpublished work). This toxoid induces significant titers of neutralizing and precipitating antibodies for toxin A in several species of animals when given with appropriate adjuvants. The purpose of this study was to test the purified toxoid of Ps. aeruginosa in a well characterized model of burn wound infection in the rat. For comparison, groups of animals actively immunized with live Ps. aeruginosa were also studied.

MATERIALS AND METHODS

Animals - Adult Sprague-Dawley (Holtzman strain) rats were used throughout this investigation. The rats were conditioned on Wayne's Lab

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1. Callahan LT: Purification and characterization of Pseudomonas aeruginosa. III. Characteristics of antitoxin. Am J Infect Dis 128: 520-526, 1973.
 3. Leppla SH: Large-scale purification and characterization of the exotoxin of Pseudomonas aeruginosa. Infect Immun 14: 1077-1086, 1976.
 2. Iglewski BH, Liu PV, Kabat D: Mechanism of action of Pseudomonas aeruginosa exotoxin A: Adenosine diphosphateribosylation of malian elongation Factor 2 in vitro and in vivo. Infect Immun 15: 138-144, 1977.

Blox and water ad libitum for at least two weeks prior to any experiment. The weight range of individual rats was within ± 10 gm of the average weight for the group. At the start of the immunization period the rats weighed 100 gm. Weights at the time of burn and challenge ranged from 350 to 370 gm.

Experimental and control animals were anesthetized with sodium pentobarbital (1mg/25 gm) prior to burning. Burns were inflicted on the dorsum after clipping the hair with a No. 40 blade in an Oster animal clipper. Each animal was placed in a fixed-area shield (6) and the dorsum was immersed in boiling water for 10 seconds. This procedure produces a uniform full-thickness burn covering 18 to 22 percent of the total body surface.

After burning, water and food were provided ad libitum for all test animals. An ambient temperature range of 21-27°C was maintained. Survivors were observed for 30 days after inoculation.

Organisms - Test organism Ps. aeruginosa (ISR 12-4-4(59)) and ISR 8-28-3(63) were the challenge microorganisms employed throughout the study. These microorganisms were originally isolated from burn patients who expired with septicemia, and are known to be virulent in this model. A sufficient quantity was grown on Trypticase Soy Agar to be used for the entire series of experiments. After 18 to 24 hours of incubation, the microorganisms were harvested and added to sterile evaporated milk. Two ml aliquots of this mixture were placed in sterile 5 ml bottles. The bottles were sealed with sterile rubber gaskets and aluminum caps. This sealed bottle procedure prevented contamination and simplified the removal of bacterial suspension. The sealed bottles were quick-frozen and placed in storage at -80°C for preservation.

Cultures for use in the experiments were prepared from the frozen, preserved samples; 0.1 ml of the microorganism-milk mixture was placed in 9.9 ml Trypticase Soy Broth and incubated for 18 hours prior to use.

Challenge Technic - The usual seeding culture contained 10^8 organisms per ml and one ml of this 18-hour culture was routinely used. The standard area topically seeded was 105 cm². The dose was evenly distributed over the entire burned area.

Immunization - Groups of animals were immunized with live Ps. aeruginosa. The immunization schedule employed for these animals was a single intraperitoneal injection of Ps. aeruginosa each week for four weeks. Immunization (5) was initiated with 0.02 ml of an 18-hour culture of Ps. aeruginosa; the second dose was 0.05 ml, the third, 0.1 ml and the fourth, 0.2 ml. One group of animals was immunized with toxoid. The immunization

6. Walker HL, Mason AD, Jr: A standard animal burn. J Trauma 8: 1049-1051, 1968.

5. Walker HL, Mason AD, Jr, Raulston GL: Surface infection with Pseudomonas aeruginosa. Ann Surg 160: 197-305, 1964.

technic employed for these animals was a single 0.5 ml intramuscular dose of toxoid each week for four weeks.

Immunization Control - A second group of animals were injected with aluminum phosphate, using the same injection dose and technic as that employed for toxoid.

RESULTS

Active Immunization with Ps. aeruginosa ISR Strains 12-4-4(59) and 8-28-3(63) - Table 1 summarizes the results of immunization and challenges. Twenty-four animals were immunized with ISR strain 12-4-4(59) and challenged with the standard burn procedure. In the first group of 17 animals immunized with ISR strain 12-4-4(59) and challenged with ISR strain 12-4-4(59), no deaths occurred during the 30 day period of observation. The second group of seven animals was immunized with ISR strain 12-4-4(59) and challenged with ISR strain 8-28-3(63). All died between days 9 and 17. Twenty-two animals were immunized with ISR strain 8-28-3(63) and 12 were challenged by the standard procedure using ISR strain 8-28-3(63). In this group of 12 animals, no deaths occurred during the 30-day period of observation. The second group of 10 animals immunized with ISR strain 8-28-3(63) were burned and challenged with ISR strain 12-4-4(59) and all died between days 9 and 17.

Antitoxic Immunization with Toxoid A - Thirty-nine animals were immunized with toxoid A and challenged with the standard burn procedure and ISR strain 12-4-4(59) or ISR strain 8-28-3(63). In the first group of 20 animals burned and challenged with ISR 12-4-4(59), all died between days 6 and 18. The second toxoid immunized group of 19 animals were challenged with the standard burn procedure and ISR strain 8-28-3(63). All the animals in this group died between days 5 and 12.

Aluminum Phosphate Injections - Forty-one immunization control animals were injected with aluminum phosphate and challenged with the standard procedure and ISR strains 12-4-4(59) or 8-28-3(63). In the first group of 22 animals challenged with ISR strain 12-4-4(59), all died between days 7 and 15. A second group of 19 animals were challenged with the standard burn procedure and strain ISR 8-28-3(63), and all died between days 5 and 17.

Burn Challenged Controls - Twenty-one control animals were challenged with the standard burn procedure and ISR strain 12-4-4(59). All died between days 6 and 17. A second group of 20 animals were challenged with the standard burn procedure and ISR strain 8-28-3(63). All died between days 5 and 11.

Pathology - Selected rats from each test group were examined for gross and microscopic lesions. Pathologic changes were similar in all groups that died.

The dorsal burn wounds were edematous and hemorrhagic at the junction

Table 1. Summarized results of immunizations and challenges

Immunization and Challenge	No. of Animals	No. Died	Time to death Days (mean)	Volume of 18-Hr. Culture (ml)	Strains of Pseudomonas
Live 14-4-4(59) challenged with 12-4-4(59)	17	0	0	1.0	12-4-4(59)
Live 12-4-4(59) challenged with 8-28-3(63)	7	7	9-17 (12.1)	1.0	12-4-4(59) 8-28-3(63)
Live 8-28-3(63) challenged with 8-28-3(63)	12	0	0	1.0	8-28-3(63)
Live 8-28-3(63) challenged with 12-4-4(59)	10	10	9-17 (12.8)	1.0	8-28-3(63) & 12-4-4(59)
Toxoid absorbed aluminum phosphate challenged with 12-4-4(59)	20	20	6-18 (10.6)	1.0	12-4-4(59)
Toxoid absorbed aluminum phosphate challenged with 8-28-3(63)	19	19	5-12 (7.6)	1.0	8-28-3(63)
Aluminum phosphate adjuvant challenged with 12-4-4(59)	22	22	7-15 (10.2)	1.0	12-4-4(59)
Aluminum phosphate adjuvant challenged with 8-28-3(63)	19	19	5-17 (8.3)	1.0	8-28-3(63)
Burned challenged controls (12-4-4(59)	21	21	6-17 (10.3)	1.0	12-4-4(59)
Burn challenged controls (8-28-3(63))	20	20	5-11 (7.5)	1.0	8-28-3(63)

of normal and burned skin. Suppuration and necrosis were seen at the eschar base after one week. Invasive bacterial infection was found in all skin sections examined histologically. Focal hemorrhagic and necrotic lesions were seen in various organs including the lung, kidney, spleen and liver, and occasionally intestine. Microscopically, all the hemorrhagic and necrotic lesions were found to contain gram-negative rod-shaped bacteria. A pattern of necrotic bacterial vasculitis typical of Ps. aeruginosa was seen in many of the skin and hematogenous lesions. Ps. aeruginosa was cultured from lung, liver and spleen in these animals.

DISCUSSION

These experiments demonstrate that immunization with aluminum phosphate absorbed toxoid prepared from *Pseudomonas* exotoxin failed to protect the septic burn rat model. Previous experiments (5) have shown that active immunization utilizing Ps. aeruginosa provides good strain-specific protection in the same infection model. Those experiments showed no cross protection between strains of Ps. aeruginosa. This study confirms that active immunization utilizing live Ps. aeruginosa provides strain specific protection in this model and no cross protection. Deaths were consistently caused by invasive infection and hematogenous spread of the infection to various organs including the lung, kidney, spleen and heart. These inflammatory lesions do not occur in the burned mouse model (4) suggesting that the latter may be a toxic rather than an infectious model.

PUBLICATIONS AND/OR PRESENTATIONS

None

5. Walker HL, Mason AD, Jr, Raulston GL: Surface infection with *Pseudomonas aeruginosa*. *Ann Surg* 160:197-305, 1964.

4. Stieritz DD, Holder IA: Experimental studies of the pathogenesis of *Pseudomonas aeruginosa* infection: Evidence for in-vivo production of a lethal toxin. *J Med Microbiol* 11:101-109, 1978.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

**REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- MULTIPLE ANTI-
BIOTIC RESISTANT SERRATIA MARCESCENS ISOLATED
FROM BURN PATIENTS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234**

1 October 1977-30 September 1978

Investigators:

**Virginia C. English, MS
Robert B. Lindberg, PhD**

Reports Control Symbol MEDDH-288 (R1)

Unclassified

ABSTRACT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

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US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 October 1977-30 September 1978

Investigators: Virginia C. English, MS
Robert B. Lindberg, PhD

Reports Control Symbol MEDDH-288(R1)

Antibiotic sensitivity on 287 strains of *Serratia marcescens* from burn patients was assessed by a modified Kirby-Bauer technic. Kanamycin, cephalothin, ampicillin, chloramphenicol, and tetracycline were each relatively ineffective, with only a few isolates sensitive. Polymixin B inhibited 81.2% of strains, but resistant colonies were consistently observed in the inhibition zone. Strains were resistant to nitrofurantoin and to sulfadiazine. Fewer than 50% of strains were gentamicin-sensitive. Bacteremic strains were concentrated in a limited number of phage types, but existence of specific bacteremic types could not be confirmed. Long term variation in intensity of *Serratia* colonization in the burn ward were demonstrated.

Serratia
Infection
Burns
Septicemia
Humans
Antibiotic

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- MULTIPLE ANTIBIOTIC
RESISTANT *SERRATIA MARCESCENS* ISOLATED FROM
BURN PATIENTS

Bacterial sepsis remains a major problem and a significant cause of death. Gram negative organisms of the *Enterobacteriaceae* group are a prominent part of the burn wound flora and are frequently recovered from blood cultures of septic patients. Antibiotic therapy is the only significant means of coping with established sepsis, and the antibiotic sensitivity of prominent offending bacterial species is a critical part of such an approach.

At the Institute of Surgical Research (ISR) major emphasis is placed upon antimicrobial testing of strains recovered from blood culture. Few organisms from other sources have been studied by antibiogram. This report is concerned with an in depth study of antimicrobial susceptibility testing of *Serratia marcescens* recovered from burn patients.

Selection of *S. marcescens* as an appropriate bacterial species for this study was prompted by several facts: (a) *S. marcescens*, an opportunistic pathogen in the compromised patient (1,2,3), has been recovered with relative frequency from burn patient cultures representing a variety of sources; (b) in three consecutive years there occurred at ISR a noticeable increase in the number of patients with *Serratia*-positive blood cultures on one or more occasions; (c) there has been developed in this laboratory a bacteriophage typing system for *Serratia* (4). This system allowed precise determination of strain identity of each *Serratia* isolate recovered from patients hospitalized on the burn ward. Utilizing the phage system the epidemiologic pattern of *Serratia* has been monitored since 1968.

MATERIALS AND METHODS

Serratia from burn patient cultures were re-isolated on blood agar plates (BAP) after 24 hour incubation at 37°C. An isolated colony was selected and stabbed into 6 ml of Trypticase Soy Agar (TSA) in a 13 x 100 mm test tube. Inoculation was made in the center of the agar column, thrust almost but not quite to the bottom of the tube. These stored cultures were incubated

1. Altmeier WA, Culbertson WR, Fuller WD, McDonough J: *Serratia marcescens* septicemia. Arch Surg 99: 232-238, 1968.
2. Cardos SF, Florman AL, Simberkoff MS, Lanier L: *Serratia marcescens*: Use of detailed characterization of strains to evaluate an increase of isolates in an intensive care unit. Am J Med Sci 266: 447-452, 1973.
3. William TW, Jr, Sailer JE, Virosław J, Knight V, Glasgow J, Moreland N: *Serratia marcescens* as a postoperative pathogen. Am J Surg 122: 64-73, 1971.
4. English VE, Latta RL, Brame RE, Lindberg RB: Development of a bacteriophage typing system for organisms of the genus *Serratia*. USA Surg Res Unit Ann Rpt FY 1968. Brooke Army Medical Center, Fort Sam Houston, Texas. Section 32.

overnight at 37°C, the slip cap of the tube removed, and the tube sealed with a cork stopper impregnated with Vaspar (vaseline-paraffin mixture). The cultures were stored in the dark at room temperature until sensitivities were performed.

Antimicrobial susceptibility testing was performed using a standardized disc diffusion method endorsed by the Federal Drug Administration (FDA) (5) and the National Committee for Clinical Laboratory Standards (NCCLS) (6). A modification of the method described by Bauer involved inoculation of the test plate by an agar overlay technic (7). Zones of inhibition obtained by using the agar overlay technic of inoculation has been shown to compare favorably to those of the Kirby-Bauer method (8).

Four to five colonies of a pure culture were selected from a BAP and transferred to a 0.5 ml of Brain Heart Infusion (BHI) broth. The cultures were incubated 4 hours in a 36°C water bath. A calibrated loop of 0.001 ml capacity was used to seed 9 ml of melted and cooled 1.5% agar. The seeded agar solution was gently mixed and poured over the surface of Mueller-Hinton Agar contained in a 150 mm petri dish. Antibiotic discs listed in Table 1 were dispensed onto the solidified overlay with a BBL Sensi-dispenser. The plates were inverted and incubated overnight at 37°C. After incubation, zone diameters were measured using a Fisher-Lilly zone reader. Organisms were designated as resistant, intermediate or sensitive in terms of each antibiotic and the zone sizes assigned to it by the manufacturer.

RESULTS

Susceptibility testing of *S. marcescens* to antimicrobial chemotherapeutic agents was performed on 287 isolates collected from February 1974 through August 1978. The pattern in which Serratia occurred was consistent to that noted in the past at ISR (9). When Serratia appeared on ward patients, the event was abrupt and colonization of patients reached epidemic proportions rapidly. Epidemic episodes generally lasted from one to three months, after which the number of Serratia recovered from patients fell dramatically. An

5. Federal Register. Rule and Regulations. Antibiotic Susceptibility Discs. 37: 20525-20529, 1972.

6. Balows A, Hall CT, Gavan TL: Standardization and quality control of disc susceptibility testing in the United States. In Bondi A, Bartola JT, Preer JE (eds). The Clinical Laboratory as an Aid in Chemotherapy of Infectious Disease. Baltimore, Univ Park Press. 1977, pp.29-43.

7. Matsen JM, Barry AL: Susceptibility testing: Diffusion procedures. In Lennette EH, Spaulding, Truant (eds). Manual of Clinical Microbiology. 2nd ed. Washington, Am Soc for Microbiol. 1974, pp.418-427.

8. Barry AL, Garcia F, Thrupp LD: An improved single-disk method for testing the antibiotic susceptibility of rapidly growing pathogens. Am J Clin Path 53: 149-158, 1970.

9. English VE, Lindberg RB, Mason AD, Jr, Pruitt BA, Jr: Bacteriophage types of *Serratia marcescens* from burn wounds of military personnel. USA Inst Surg Res Ann Rpt FY 1974. BAMC, Fort Sam Houston, Texas. Section 12.

Table 1. Agents Used in Testing Serratia marcescens for Sensitivity

Chemotherapeutic Agent	Disc Potency (ug)
Amikacin	10
Ampicillin	10
Cephalothin	30
Chloramphenicol	30
Gentamicin	10
Kanamycin	30
Nalidixic acid	30
Nitrofurantoin	300
Polymixin B	300 U
Sulfadiazine	250
Tetracycline	30

intervening period with no *Serratia* encountered would end with the next outbreak of the organism. Figure 1 summarizes the epidemiologic pattern of *Serratia* from 1974-1978. The results of susceptibility tests performed on the collection of *Serratia* isolates are summarized in Table 2. All isolates, with one exception, were sensitive to amikacin. The resistant strain, recovered from sputum in February 1976, gave no zone of inhibition on repeated testing. It was sensitive to all other drugs tested except cephalosporin. The strain was of the phage type designated K, and it was the only strain of type K recovered.

Gentamicin has been used since 1971 to treat burn patients at ISR. By 1974, some resistance of *Serratia* to that drug had developed (Fig. 2). However, 61.2% of *Serratia* isolates collected that year were sensitive to the drug. In 1975, 158 isolates of *Serratia* were recovered and only 49 (31.0%) of those were found to be sensitive to gentamicin. In the early months of 1975, when few isolates were recovered, sporadic urine and sputum cultures yielded gentamicin-resistant *Serratia* (Table 3).

During September, October and November, when *Serratia* appeared in epidemic numbers, the proportion of resistant strains rose markedly. Such resistant strains were recovered from wounds, sputum, intravenous sites and blood, as well as from postmortem samples of wound viscera and blood. Not infrequently, a patient harbored *Serratia* of different phage types. Recovery of multiple phage types had been reported previously in burn patients with *Pseudomonas aeruginosa* (10). One patient colonized with *Serratia* on several body sites showed strain and sensitivity changes which merited detailed

10. Latta RL, Lindberg RB, Pruitt BA, Jr, Mason AD, Jr: Bacteriophage types of *Pseudomonas aeruginosa* found in burned soldiers. USA Inst Surg Res Annual Rpt FY 1973. BAMC, Fort Sam Houston, Texas. Section 12.

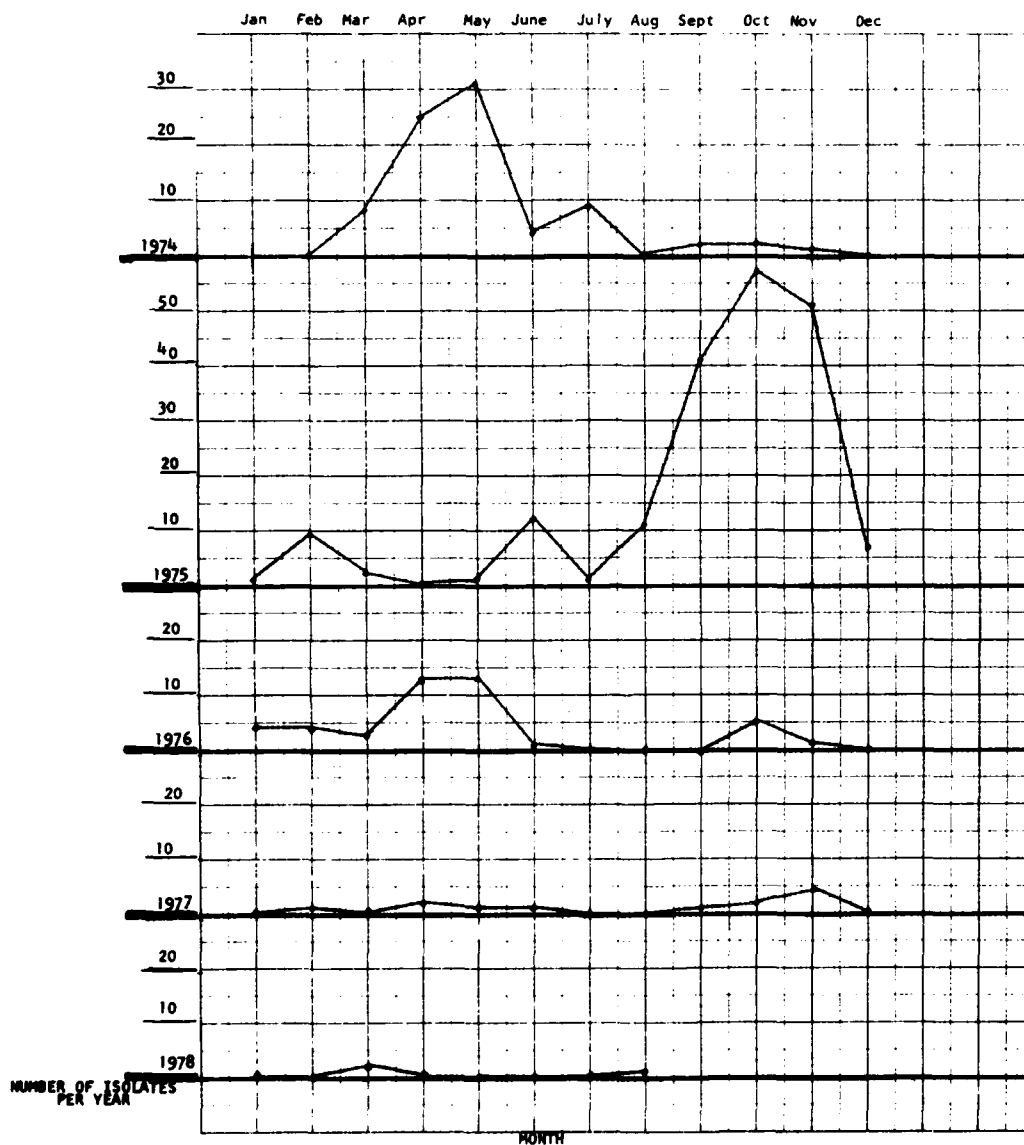


Fig. 1. Annual incidence of Serratia in burn patients.

Table 2. Antimicrobial Susceptibility Test Results for 287 Strains of *Serratia marcescens*

Antibiotic	S*	1974		R+	S	1975		S	1976		S	1977		S	I	1978		Total	
		S*	I*			R	R		R	R		S	I			S	I	R	
Kanamycin	29	0	51	40	0	118	16	0	19	7	0	2	5	0	0	97	0	190	
Nitrofurantoin	19	21	40	17	41	100	12	8	15	3	2	4	1	3	1	52	75	160	
Cephalothin	0	0	80	2	0	156	0	1	34	0	0	9	0	0	5	2	1	204	
Ampicillin	2	4	74	3	0	155	2	1	32	1	1	7	0	0	5	8	6	273	
Chloramphenicol	49	0	31	41	2	115	26	0	9	7	0	2	5	0	0	128	2	157	
Polymyxin B	53	1	26	145	1	12	24	2	9	6	0	3	5	0	0	233	4	50	
Sulfadiazine	44	2	34	33	7	118	17	0	18	5	0	4	5	0	0	104	9	174	
Gentamicin	49	1	30	49	0	109	20	0	15	8	0	1	5	0	0	131	1	155	
Nalidixic acid	77	2	1	119	34	5	28	0	7	8	1	0	5	0	0	237	37	13	
Tetracycline	44	23	13	34	104	20	15	13	7	1	3	5	1	3	1	95	146	46	
Amikacin	80	0	0	158	0	0	34	0	1	9	0	0	5	0	0	286	0	1	

* S = Sensitive strains
I = Intermediate strains
R = Resistant strains

LEGENDS FOR ANTIBIOTICS SHOWN IN FIGURE 2.

K = Kanamycin

FM = Nitrofurantoin

CF = Cephalothin

AM = Ampicillin

AN = Amikacin

C = Chloramphenicol

PB = Polymixin B

SD = Sulfadiazine

GM = Gentamicin

NA = Nalidixic acid

TE = Tetracycline

Table 3. Gentamicin Resistant Serratia Strains
1975

Month	Source	No. of Resistant Isolates
January	-	-
February	Urine	2
March	Lukens	1
April	-	-
May	-	-
June	Lukens	2
	Urine	1
July	-	-
August	Lukens	4
	Urine	2
September	Lukens	7
	Blood	3
	Postmortem	17
October	Lukens	13
	Urine	7
	Blood	7
	IV sites	1
	Postmortem	6
November	Lukens	14
	Surface areas	2
	Blood	8
	IV sites	1
	Urine	6
	Postmortem	1
December	Lukens	3
	Urine	1

presentation (Table 4). On October 2, *Serratia* was first isolated from bronchial secretions. The strain was phage type A-13, and gentamicin resistant. An identical strain was recovered from a lukens tube 12 days later. Subsequently, three successive blood cultures grew gentamicin resistant *Serratia* type A-13. A gentamicin sensitive isolate of phage type A-13 was recovered from tracheal secretions on October 23. No gentamicin *Serratia* of type A-13 were recovered from blood. During November, *Serratia* of phage type A-10 were recovered repeatedly from tracheal aspirates. One *Serratia* of type A-10 was later recovered from blood cultures. All isolates of type A-10 were gentamicin resistant. Concomitant with the recovery of type A-10 in November, type A-11 was recovered from the burn wound. Two days later this gentamicin resistant phage type was recovered from blood culture. During November transient phage types C-4, A-12, A-5, A-14 and A-9 were also recovered from the patient. Culture sites included lukens tube contents, Foley catheter tip and IV sites. Each of those *Serratia* phage types was gentamicin sensitive. None was isolated from blood culture.

The occurrence of *Serratia* from burn ward patients did not reach epidemic proportions in 1976. Gentamicin sensitive isolates rose to 57.1% of those recovered. The number of *Serratia* isolated from patients in 1977 and 1978 was smaller than was seen in the previous three years.

The sensitivity of *Serratia* to kanamycin fluctuated in a pattern not unlike that of gentamicin, but fewer *Serratia* isolates were sensitive to kanamycin than to gentamicin. From 1974-1978, 97 of 287 (33.8%) isolates of *Serratia* were found to be sensitive to kanamycin.

Chloramphenicol and the tetracycline group of drugs are rarely employed as therapeutic agents for burn patients at ISR. The yearly percentage of *Serratia* isolates sensitive to chloramphenicol ranged from 25.9% to 76.0%. Of the 287 *Serratia* tested in the five years studied 44.5% were sensitive to chloramphenicol. *Serratia* tested with tetracycline were designated as intermediate in reaction. In 1974, 55.0% of the *Serratia* isolates were sensitive to the tetracycline group. In 1975, 65.8% of the isolates showed intermediate sensitivity and only 21.5% were sensitive. Sensitive *Serratia* isolates in 1976 rose to 42.9% but 37.1% were of the intermediate designation. Overall, 50.9% of the *Serratia* isolates showed intermediate sensitivity reactions. Tetracycline was the only drug tested in which the size of the inhibitory zone did not produce a clearcut sensitive or resistant result for most strains of *Serratia* tested. Zones of inhibition obtained with tetracycline were precise, distinct and easily read.

Polymixin B was totally ineffective in inhibiting *Serratia*. Zones were large, distinct, clearly demarcated and easily read. However, several colonies were present within each zone of inhibition. According to Bauer, et al (11) this is an uncommon occurrence, but the growth of such colonies within the zone are considered indicative of resistance.

11. Bauer AW, Kirby WM, Sherris JC, Turk M: Antibiotic susceptibility testing by a standardized single disc method. *Am J Clin Path* 45: 493-496, 1966.

Table 4. Gentamicin Sensitivity to *Serratia* Isolated
from a Patient Harboring Several Phage Types

Phage Type	Date	Source	Sensitivity Reaction*
A-13	10- 20-75	Tracheal aspirate	R
	10-14-75	Lukens	R
	10-21-75	Blood	R
	10-22-75	Blood	R
	10-23-75	Lukens	S
	10-23-75	Blood	R
A-10	11- 1-75	Lukens	R
	11- 5-75	Lukens	R
	11- 6-75	Lukens	R
	11- 7-75	Lukens	R
	11-10-75	Lukens	R
	11-12-75	Lukens	R
	11-13-75	Lukens	R
	11-24-75	Lukens	R
	11-24-75	Blood	R
	11-25-75	Lukens	R
	11-28-75	Lukens	R
A-11	11- 7-75	Post-debridement area	R
	11- 9-75	Blood	R
C-4	11-11-75	Lukens	S
A-12	11-13-75	Lukens	S
A-5	11-16-75	Foley catheter tip	S
A-14	11-16-75	IV site	S
A-9	11-18-75	Lukens	S

* R = Resistant
S = Sensitive

Serratia isolates from all sources were tested with nalidixic acid, nitrofurantoin and sulfadiazine. Susceptibility results for these drugs are shown on a yearly basis in Table 5. Nalidixic acid inhibited 237 of 287 *Serratia* isolates tested. Thirty-six of 37 strains isolated from urine were found to be sensitive to the drug. Furadantoin inhibited the growth of 53 of 287 *Serratia* strains tested. Ten sensitive isolates from 37 *Serratia* recovered from urine were found. *Serratia* isolates recovered from urine cultures were sensitive to sulfadiazine in only 7 of 37 strains. Isolates of *Serratia* recovered from all body sources were sensitive to sulfadiazine in 104 of 287 strains. Bacterial colonization of patients at ISR is seldom restricted to one body area. Patients are most often colonized with *Serratia* in several sites, and for that reason nalidixic acid, nitrofurantoin and sulfadiazine directed primarily toward urinary tract infection, are of little value in our patients. *Serratia* resistance to cephalothin and ampicillin was 99.0% and 95.1% respectively, of those strains tested.

DISCUSSION AND SUMMARY

The epidemiologic pattern of *S. marcescens* isolated from patients in the burn wards at ISR has been tracked by bacteriophage typing. In this study, the antibiogram offered an added parameter of measurement. The recovery of *Serratia* from burn ward patients followed a pattern in which microepidemics appeared abruptly, maintained peak levels for short periods of time and then fell rapidly to valley levels for an unpredictable length of time. When colonization of patients reached epidemic proportions, individual patients were most often found to harbor organisms at multiple anatomical sites including blood.

From February 1974 to August 1978 inclusive, kanamycin, cephalothin, ampicillin, chloramphenicol and tetracycline each inhibited the growth of a small percent of the isolates tested by disc diffusion susceptibility. In all strains of *Serratia* there occurred total resistance to Polymixin B. *Serratia*, particularly those isolated from urine, were little sensitive to nitrofurantoin and sulfadiazine, but nalidixic acid was an effective inhibitory agent. It is, however, of little clinical value for the patient where bacterial colonization is not limited to urinary tract infections. Only 45.6% of the organisms were gentamicin sensitive as determined by standardized disc diffusion testing. Observation of the increasing resistance of *Serratia* to gentamicin, particularly in a burn patient population, gives cause for concern. *S. marcescens* is most often sensitive to gentamicin (12-14). However, the emergence of gentamicin

12. Cooksey RC, Bannister ER, Farrar WE, Jr: Antibiotic resistance patterns of clinical isolates of *Serratia marcescens*. *Antimicrob Agents & Chemo.* 7: 396-399, 1975.

13. Bruun JN, Mulholland SG: Antibiotic sensitivity of isolates from nosocomial and community-acquired urinary tract infection. *Urology* 1: 409-413, 1973.

14. Crowder JC, Gilkey GH, White AC: *Serratia marcescens* bacteremia. *Arch Intern Med* 128: 247-253, 1971.

Table 5. Sensitivity of *Serratia marcescens* to Nalidixic Acid, Nitrofurantoin and Sulfadiazine, 1974-1978

Source	No. Isolates	Antibiotic	Sensitive	Number of Isolates	
				Intermediate	Resistant
IV sites	7	NA* FM** SD***	4 1 3	2 0 0	1 6 4
Surface areas	40	NA FM SD	34 14 21	5 10 2	1 16 17
Sputum/lukens, Tracheal aspirates	90	NA FM SD	65 14 35	18 20 1	7 56 54
Blood	29	NA FM SD	27 6 5	0 7 1	2 16 13
Postmortem: Surface Viscera, blood	76	NA FM SD	65 5 31	9 23 2	2 48 43
Foley catheter tip	6	NA FM SD	4 2 2	2 2 1	0 2 3
Urine	37	NA FM SD	36 10 7	1 12 2	0 15 28
Eye	2	NA FM SD	2 1 0	0 1 0	0 0 2
Total	287	NA FM SD	237 53 104	37 75 9	13 159 174

* NA - Nalidixic Acid ** FM - Nitrofurantoin *** SD - Sulfadiazine

resistant S. marcescens has been reported. Meyer, et al (15) found 50% of Serratia isolates from a general hospital were gentamicin resistant. Should resistance of S. marcescens to gentamicin persist, amikacin or other aminoglycoside drugs will, of necessity, become drugs of choice in coping with epidemic outbreaks of Serratia. Serratia strains in a hospital environment have been shown to have increased resistance to amikacin during therapy (16).

At the time when patients harbored multiple antibiotic resistant Serratia of certain bacteriophage types, there was an increase in the number of Serratia bacteremias. This was most evident when the strains were resistant to gentamicin. This suggests that strains capable of blood stream invasion may in some way be related to particular phage type strains which had developed resistant drug patterns. However, a relationship of drug resistance to phage type could not be shown.

Serratia in blood stream, lung and burn wound can be lethal. Effective antibiotic agents must be readily available for clinical use if potentially lethal invasive sepsis is to be controlled. Continued monitoring of S. marcescens by susceptibility testing will dictate which chemotherapeutic agents are of clinical usefulness.

PRESENTATIONS and/or PUBLICATIONS: None

15. Meyer RD, Halter J, Lewis RP, White M: Gentamicin resistant Pseudomonas aeruginosa and Serratia marcescens in a general hospital. Lancet 1: 580-583, 1976.

16. Craven PG, Jorgensen JH, Kaspar RL, Drutz DJ: Amikacin therapy of patients with multiple antibiotic resistant Serratia marcescens infections. Am J Med 62: 902-910, 1977.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

**REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- DEVELOPMENT OF
PROPHYLACTIC TOPICAL THERAPY FOR USE ON BURN
WOUNDS OF MILITARY PATIENTS: SEARCH FOR IMPROVED
FORMULATIONS**

**US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 October 1977 - 30 September 1978

Investigators:

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Arthur D. Mason, Jr, MD**

Report Control Symbol MEDDH-288 (R1)

Unclassified

ABSTRACT

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US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
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Four new compounds, zinc-, copper-, chromium-, and cerium-sulfadiazine were synthesized and made up in 1% concentration in a water-dispersible cream base, suitable for topical application. They were applied topically on burned rats, seeded 24 to 48 hours earlier with Pseudomonas aeruginosa. Three selected virulent strains varying in invasiveness were used as test organisms, with daily topical applications of the new agents. Silver-sulfadiazine and sulfamylon treatment controls were included. Zinc-sulfadiazine and chromium-sulfadiazine were more effective than either treatment control in increasing survival. In this series only sufladiazine was used but metal-sulfamethoxazoles have already been shown to be of equivalent high potency. Copper-sulfadiazine was effective, but less so than zinc and chromium; cerium-sulfadiazine was the least effective of the preparations tried, in contrast to its reported adjunctive effect in silver-sulfadiazine.

Pseudomonas
Topical therapy
Burns
Heavy metal cation
Humans
Burn wound sepsis

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE
OF TROOPS WITH THERMAL INJURY -- DEVELOPEMENT OF
PROPHYLACTIC TOPICAL THERAPY FOR USE ON BURN WOUNDS
OF MILITARY PATIENTS: SEARCH FOR IMPROVED FORMULATIONS

Control of burn wound flora by topical therapy is now a basic part of burn treatment. The armamentarium of agents effective in control of infection in extensive burns is not large: sulfamylon and silver-sulfadiazine are the most widely used topical cream formulations, with silver-nitrate soaks and Betadine ointment less extensively used. Although resistance to sulfamylon has not appeared, silver-sulfadiazine resistance has been observed, and in any event, the topical agents now available appear to be less effective in controlling burn wound infection than they were a decade ago. Further exploration of new topical drugs appears to be warranted, since death due to sepsis is still common.

In a previous report (1), the development of zinc-, copper-, chromium- and cerium- sulfadiazines, together with metal compounds of sulfamethoxazole, sulfamethazine and sulfamylon, were described. Initial trials explored a spectrum of concentrations of these compounds, up to 10% of the material dispersed in an aqueous gel. Assay of effectiveness was carried out using the ISR burned rat model, with a 20% of body surface burn on a 200 g rat. The dorsum of the rat was clipped, scalded for 10 seconds in boiling water over the 20% body surface, and seeded within the hour with 10^8 Pseudomonas aeruginosa from a broth culture. A spectrum of challenge strains of established virulence was used for infection of the animals.

Initial results indicated that the metal-sulfonamide compounds were indeed effective against experimental Pseudomonas burn wound sepsis. Since concentrations of only 1% of the compound as a topical agent showed promise, more extensive trials of this strength appeared to be warranted. In all research directed toward topical therapy, the use of the least amount of the agent which can be effective is axiomatic. The amount of metal ion that is absorbed with these formulations has not yet been determined, but it is obvious that a minimal input is desired.

The selection of metals for this study, and the technic of preparing the metal-sulfonamide formulations were described in the 1977 annual report. More extensive studies have been necessary in order to establish a valid evaluation of the preparations. To that end, groups of animals were tested in replicate to establish the exact degree of protection that these 1% suspensions afford, and to study further the nature of variations in effectiveness which appear

1. Lindberg RB, Daye GT, Johnson AA, Pruitt BA, Jr, Mason AD, Jr: Development of topical therapy for use on burns of military patients: Search for new formulations. USA Inst Surg Res Annual Rpt FY 1977, BAMC, Fort Sam Houston, Texas. p. 273.

when several challenge strains are used. Agar diffusion tests of inhibitory action on challenge strains and on sulfadiazine-resistant strains of *P. aeruginosa* were carried out, as were tests of the inhibitory action of the four metals tested as nitrate salts.

RESULTS

A diffusion test for activity of the several metal sulfadiazine compounds was carried out. Trypticase soy agar (TSA) plates were seeded with a broth culture of the *Pseudomonas* strain, and stainless steel cylinders were placed on the plate. These were then filled with the 1% metal-sulfadiazine in the aqueous gel vehicle. Overnight incubation permitted detection of diffusion zones affecting some but not all strains tested. Table 1 presents these results. The first four strains listed were challenge strains, collected between 1959 and 1973. The remaining eight strains were collected in 1978 and were all sulfadiazine resistant. The significant aspect of these determinations is its lack of correlation with therapeutic activity. Cerium-sulfadiazine showed *in vitro* action against only one strain. Chromium-sulfadiazine was active against all four challenge strains, as was zinc-sulfadiazine. Copper-sulfadiazine was effective with 3 out of 4 virulent strains, and silver-sulfadiazine was active against all four strains. Silver-sulfadiazine, as a standard of comparison, was active against all of the strains in this series. However, as will be shown, therapeutic action with other metal-sulfonamide compounds was as good or better than that shown by silver-sulfadiazine. Lack of direct correlation between *in vitro* and *in vivo* effects has been observed with various antimicrobial agents since the beginning of the antibiotic era.

Tests with the nitrate salts of zinc, copper, chromium and cerium were made to determine the inhibitory action of these cations. This sensitivity is shown in Table 2. The least inhibitory metal was zinc. The inhibitory level, higher than the 10 mg/ml in the test protocol, was between 25 and 50 mg/ml in separate trials. The remaining three salts were all inhibitory at 1 mg/ml, with the exception of one strain which required 10 mg/ml of chromium nitrate for inhibition. As with the metal-sulfonamide tests, the metal ions showed inhibitory action that was consistent but not correlated with therapeutic efficiency.

The major effort in assessing the potential value of the new metal-sulfonamides was directed toward their activity in preventing burn wound sepsis in the burned, seeded rat model. Earlier trials has used a range of concentrations, essentially from 1% to 5%, in the aqueous gel vehicle. The conclusion was reached that survival at a significant level could be achieved with 1% concentrations of the new compounds. Comparison with 2% concentrations was made with certain compounds and challenge strains, but the essential determination in this series was the control of *Pseudomonas* burn wound sepsis with 1% metal-sulfonamides.

In this resume, the behavior of three challenge strains is presented: 12-4-4-59, 8-28-3-63, and VA-134. These strains possess different levels of virulence in the burn model; 12-4-4-59 has, in extended series, been lethal from 85% to 95% of animals, 8-23-3-63 in 93% to 96%, and VA-134 kills from

Table 1. Agar Diffusion Inhibition Zones with Metal-Sulfonamide Compounds vs *P. aeruginosa* (1)

Strain <i>P. aeruginosa</i>	Ce	Metal-Sulfadiazine 1%: Zone in mm			
		Cr	Cu	Zn	Ag
12-4-4	R ⁽²⁾	17.5 ⁽³⁾	14.1	16.9	16.9
8-28-3	R	12.9	R	12.9	12.9
VA-134	R	17.3	15.0	19.0	19.0
3-13-36	R	19.4	15.6	19.0	19.0
8-14-11	R	R	R	R	12.5
8-14-17	R	R	R	R	16.2
8-14-31	12.8	21.1	12.9	19.1	17.3
8-14-35	R	R	R	R	16.6
8-13-60	R	R	R	R	12.3
8-14-4	R	R	R	R	14.1
8-14-33	R	R	R	R	12.6
8-14-34	R	R	R	R	12.8

(1) 1% of each compound in water-dispersible gel, in stainless steel cylinder 8.6 mm diameter.

(2) R = No inhibition

(3) Zone, in mm, was the average of three trials.

Table 2. *in vitro* Sensitivity of *P. aeruginosa* Strains
to Heavy Metals: Zinc, Copper, Chromium and Cerium as nitrates

Strain <i>P. aeruginosa</i>	Minimum Inhibitory Concentration: mg/ml			
	Zn	Cu	Cr	Ce
12-4-4-59	>10	1	1	1
8-28-3-63	>10	1	1	1
VA-134	>10	1	1	1
3-13-36-73	>10	1	1	1
3-13-11-73	>10	1	1	1
6-14-30	>10	1	1	1
7-6-42	>10	1	10	1

98% to 100 % of untreated seeded rats, depending on long term fluctuations in the state of the animals. The survival results are presented as cumulative increments made up of the number of animals in each individual experiment, expressed as the percentage of animals surviving.

Figure 1 shows the behavior of zinc-sulfadiazine with *Pseudomonas* strain 12-4-4. An anomalous finding was observed: 1% zinc-sulfadiazine achieved 100% survival, while a 2% suspension was less effective. Silver-sulfadiazine as a treatment control permitted survival of approximately 80% of the animals. Sodium sulfadiazine, was also used in 1% concentration, and survival of 65% of the animals was achieved.

The same protocol with strain 8-28-3 is shown in Figure 2. Again, the 1% zinc-sulfadiazine was the most effective treatment modality; with survival in the 96% range. Silver sulfadiazine had a survival rate of 70%, and with this more virulent strain, 1% sulfadiazine saved less than 50% of animals.

The highly virulent, more rapidly invasive strain VA-134 showed a marked deviation in the survival patterns from the two preceding strains (Fig. 3). Here the 2% zinc-sulfadiazine was more effective than the 1% strength; the ultimate survival rates were 65% and 57%. Silver-sulfadiazine was only slightly more effective than sulfadiazine; the survival rate was in the range of 12% to 15%.

For sake of brevity, the other compounds are presented with all three challenge strains on a single graph. Silver-sulfadiazine and sulfadiazine

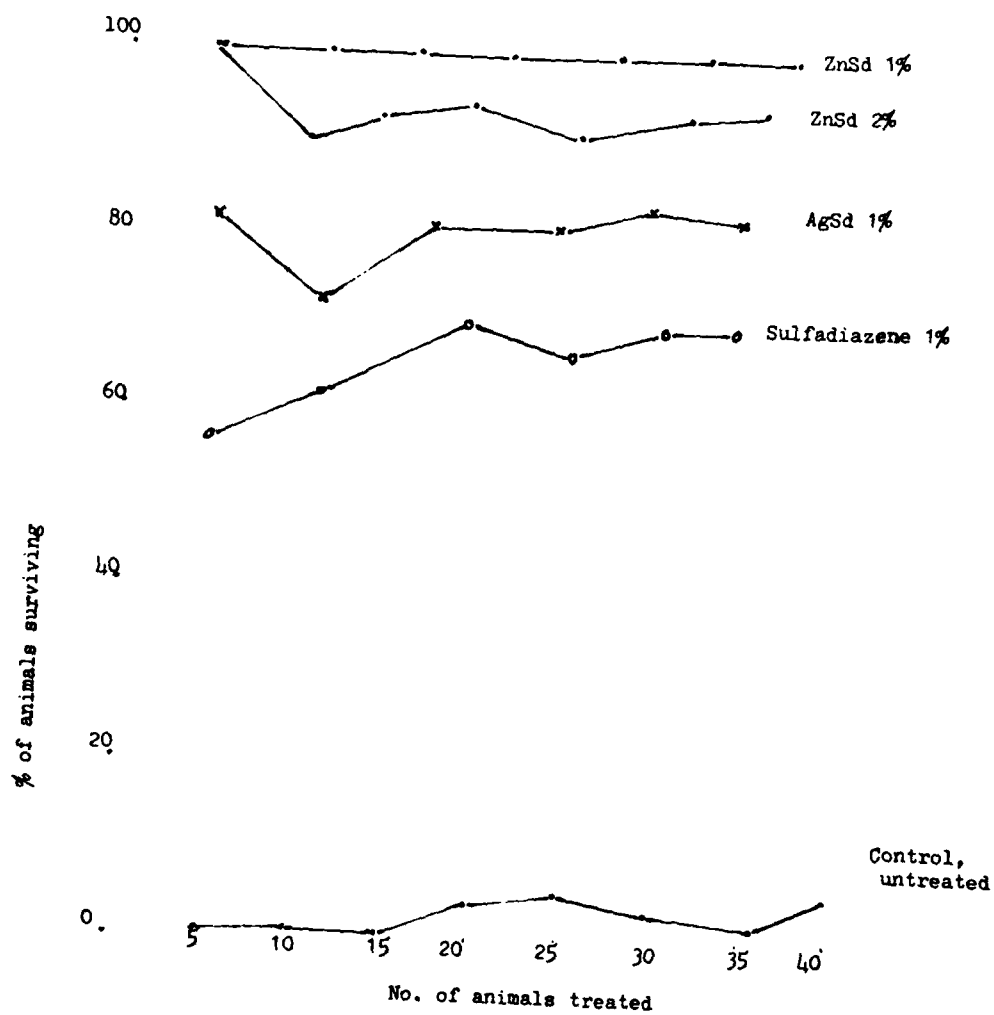


Fig. 1. Survival of burned, *Pseudomonas*-seeded rats with zinc-sulfadiazine -- Challenge strains: *P. aeruginosa* 12-4-4-59.

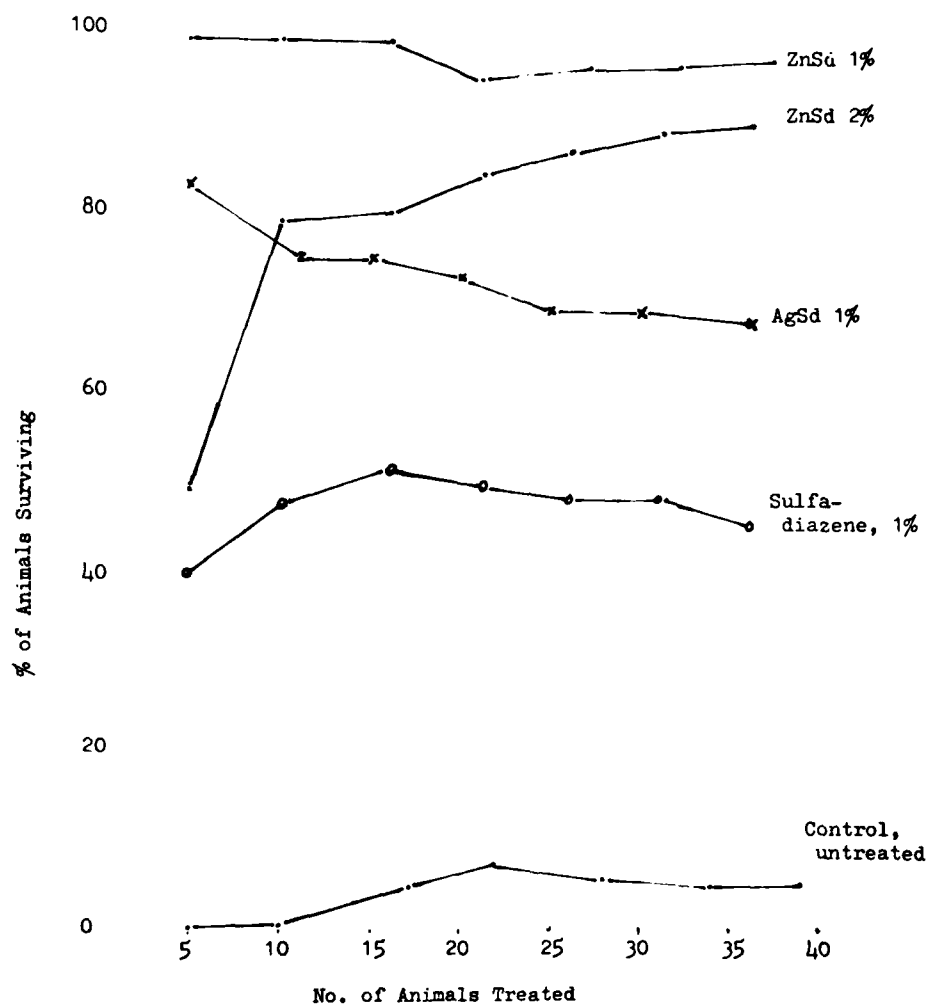


Fig. 2. Survival of burned, *Pseudomonas*-seeded rats with zinc-sulfadiazine -- Challenge strain: *P. aeruginosa* 8-28-3.

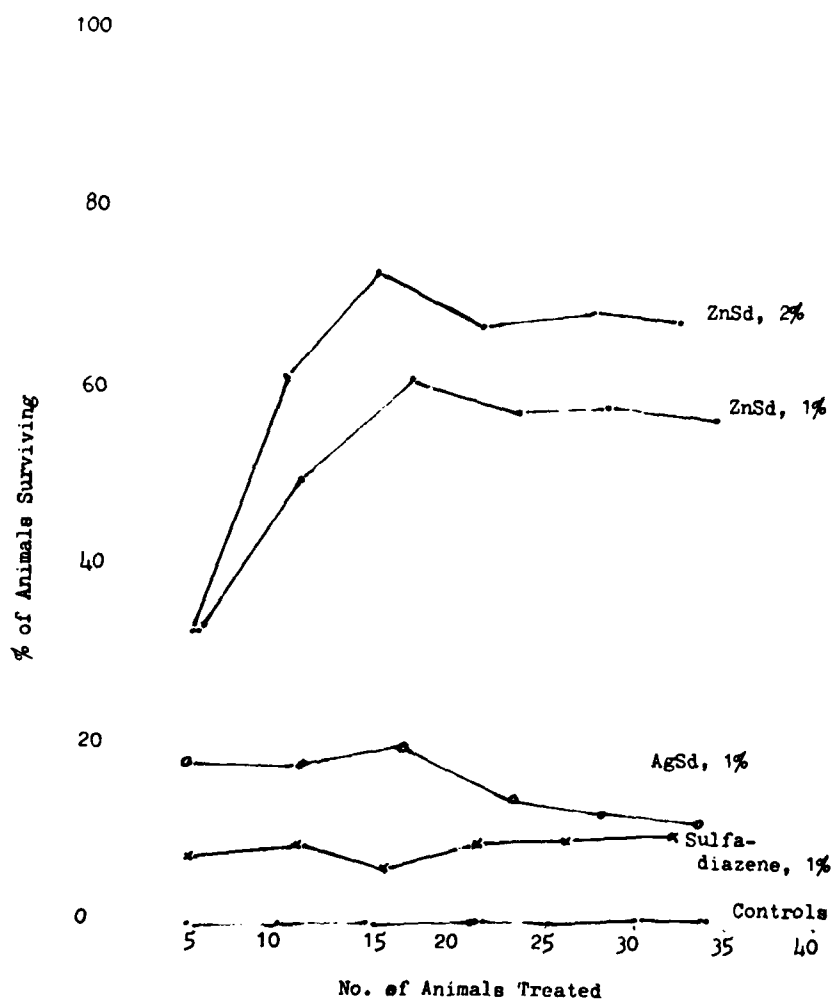


Fig. 3. Survival of burned, *Pseudomonas*-seeded rats with zinc sulfadiazine
Challenge strain: *P. aeruginosa* VA 134.

controls were run but did not differ materially from the results obtained with zinc-sulfadiazine. These values are omitted from the results with copper-sulfadiazine. This is summarized in Figure 4. The highest survival occurred with 2% copper-sulfadiazine; the 1% suspension was about 5% less effective. Survival rates with strain 12-4-4 were in the 85% to 90% range. With strain 8-28-3, 2% copper-sulfadiazine was again about 5% more effective than 1%, with survival in the range of 65% to 70%. Strain VA-134 permitted survival, as would be expected of a more virulent strain, at the 35% to 40% level, again with 2% more effective than the 1% concentration.

Chromium-sulfadiazine proved to be more effective than copper-sulfadiazine in control of experimental burn wound sepsis, and approached the performance of zinc-sulfadiazine. Figure 5 shows the results with 1% concentrations of the chromium compound, compared with the behavior of 1% silver-sulfadiazine. There was 100% survival with strain 12-4-4; silver-sulfadiazine produced a 92% survival rate. With strain 8-28-3, chromium-sulfadiazine gave a survival rate of 88%, with silver-sulfadiazine, 78% survival. As with other compounds, VA-134 was most refractory to treatment; 46% survival with the chromium and 38% with silver-sulfadiazine.

Cerium-sulfadiazine was prepared because of the reports that adding cerium to silver-nitrate cream improved the suppressant action of that drug. With cerium sulfadiazine (Fig. 6), the 1% concentration was effective in permitting survival of part of the test group. Here the relative effectiveness related to strain was altered. Survival was greatest with strain 8-28-3, followed by 12-4-4 and VA-134, which permitted the lowest survival rate. In each instance, silver-sulfadiazine was more effective than cerium-sulfadiazine. The difference was slight with strain 8-28-3; it was an 8% difference in survival with 12-4-4, and with VA-134, survival was 20% higher with silver-sulfadiazine than with cerium-sulfadiazine. It should be emphasized that the cerium compound was effective; it was just far from as effective as the other new compounds.

DISCUSSION

Multiple tests with four new metal-sulfadiazine compounds were directed toward assessing the value of a 1% concentration of each preparation in controlling experimental *Pseudomonas* burn wound sepsis. Each compound was effective to a significant degree in increasing survival with this otherwise lethal infection. In order of effectiveness, they were ranked zinc, chromium, copper and cerium sulfadiazines. Zinc- and chromium-sulfadiazines were each more effective than silver-sulfadiazine, each in a 1% concentration. One percent sulfadiazine alone was less effective than any one of the metal-sulfadiazines. Different challenge strains responded in a destructive manner: strain 12-4-4 was, in most instances, the most susceptible to treatment, but with cerium-sulfadiazine, strain 8-28-3 was most susceptible. In vitro difference in sensitivity did not explain this difference. Silver-sulfadiazine was active in vitro against many more strains than were the new compounds, but the therapeutic response did not correspond to the in vitro activity.

It appeared that metal-sulfadiazine compounds were in general effective

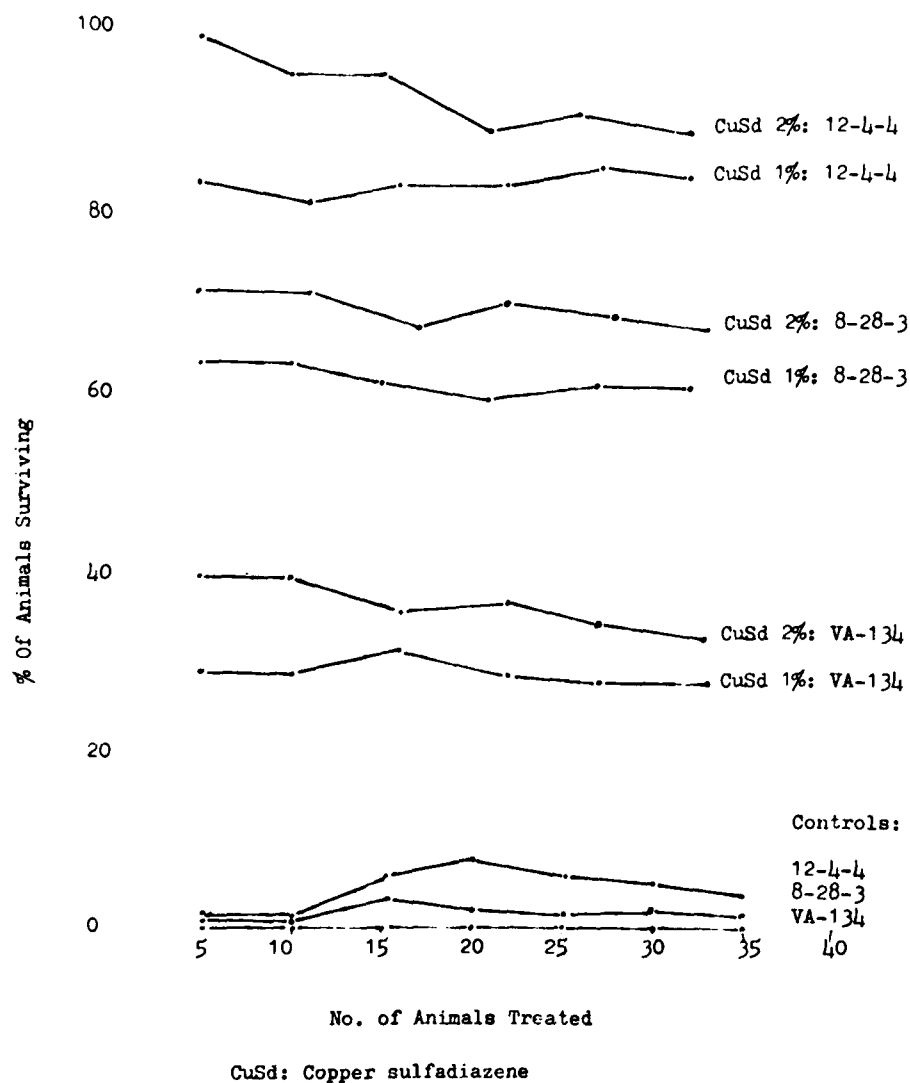


Fig. 4. Survival of burned, *Pseudomonas*-seeded rats with copper-sulfadiazene -- Challenge strains: *P. aeruginosa* 12-4-4; 8-28-3; VA-134.

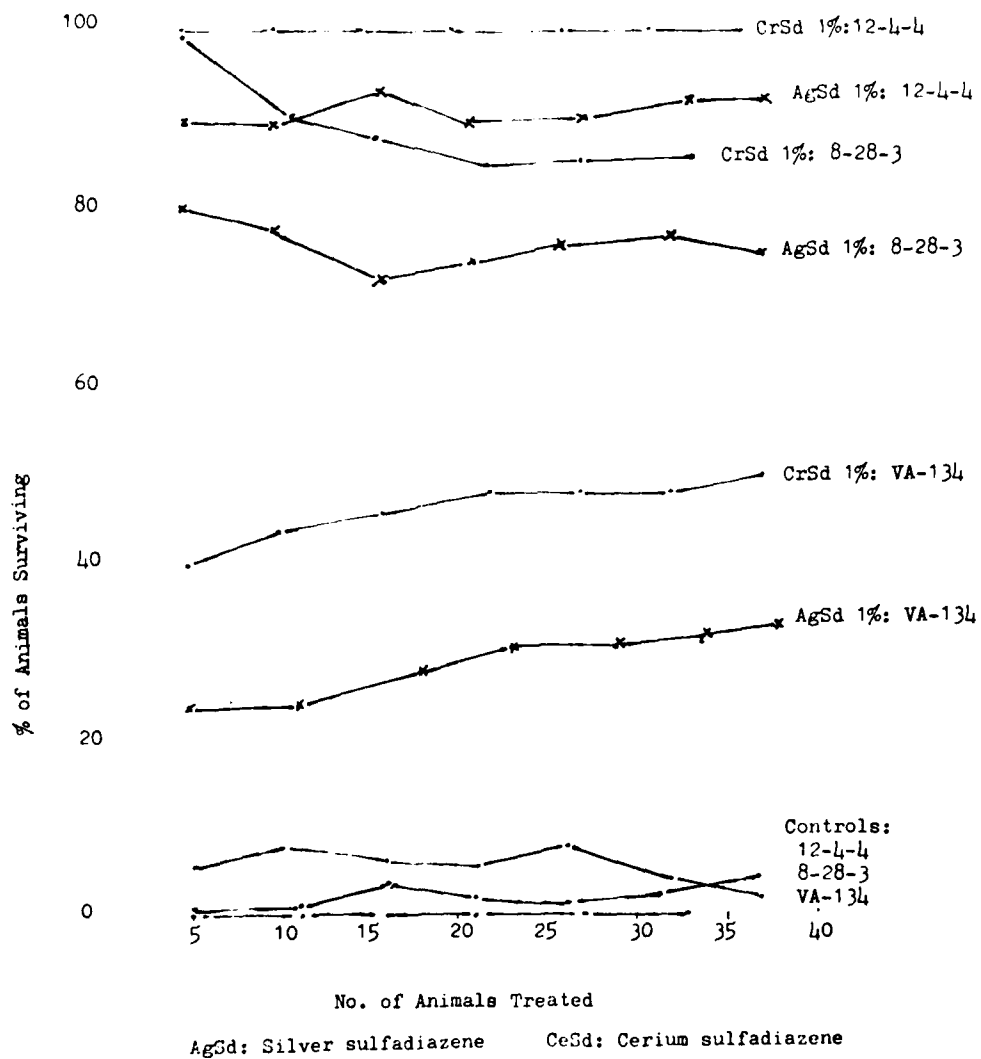


Fig. 5. Survival of burned, *Pseudomonas* seeded rats with chromium sulfadiazene. Challenge strains, *P. aeruginosa* 12 4 4, 8 28 3, VA 134.

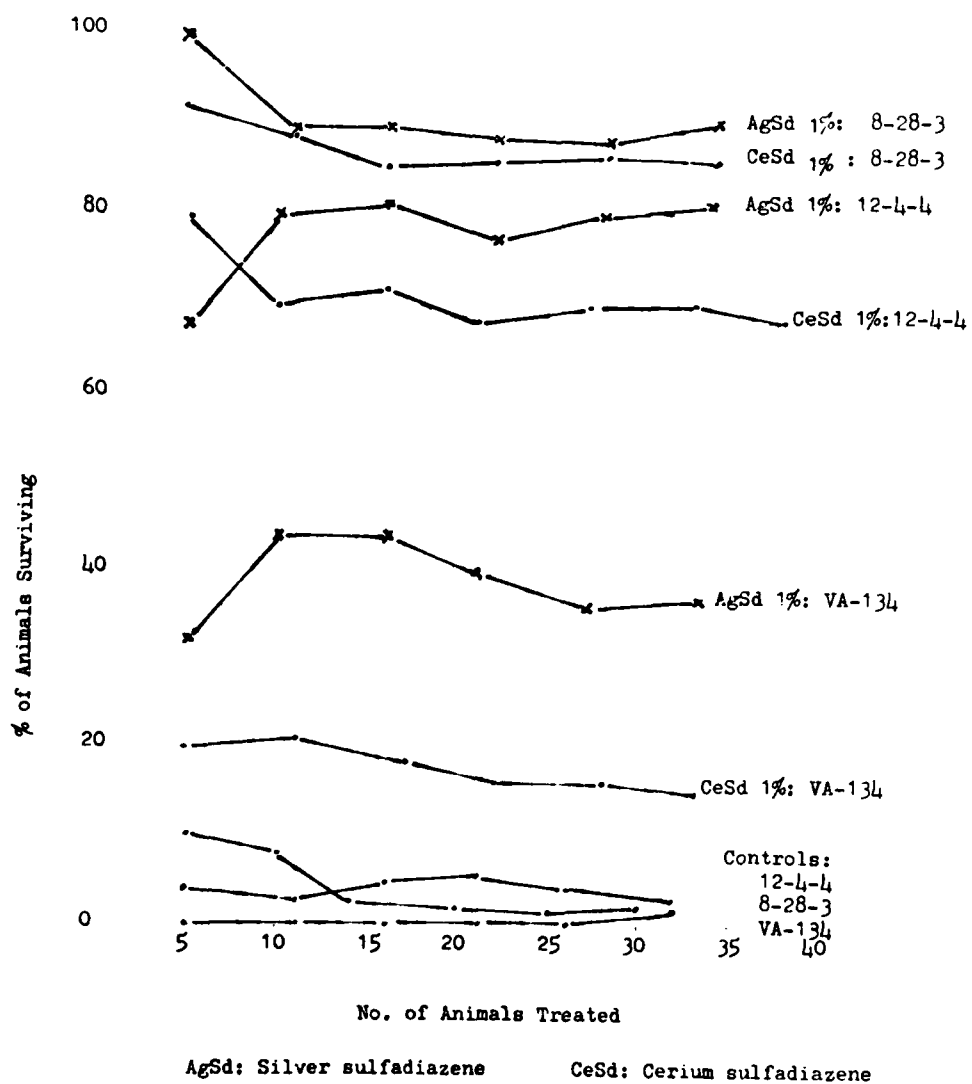


Fig. 6. Survival of burned, *Pseudomonas*-seeded rats with cerium sulfadiazene -- Challenge strains: *P. aeruginosa* 12-4-4; 8-28-3; VA-134.

in controlling burn wound sepsis. This offers the intriguing possibility that the process is not one in which both components of the compound act against the bacteria but that some protective action reflecting a fundamental difference in the action of the slightly soluble metal sulfonamide is involved.

A subsequent start has been made at investigating the behavior of mixed metal-sulfonamides. By combining two compounds half and half, the final metal concentration would be half what it was in the 1% mixture. These mixtures are effective. If this result is confirmed, the input of each metal ion could be reduced to even more negligible levels. This avenue merits exploration.

PRESENTATIONS AND PUBLICATIONS

Lindberg RB: New metal-sulfonamide compounds as topical agents in control of experimental *Pseudomonas* burn wound sepsis. Annual meeting, Am. Soc. Microbiol. 14-19 May 1978.

Lindberg RB: Control of experimental *Pseudomonas* burn wound sepsis with new metal-sulfonamide compounds. XII International Congress of Microbiology, 3-8 Sep 1978, Munich, Germany.

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SOCIAL SECURITY ACCOUNT NUMBER:									
ASSOCIATE INVESTIGATORS									
NAME: Harry R. Jacobson, MAJ, MC									
NAME:									
DA									
30. (U) Acute renal failure; (U) Renal physiology; (U) Renal pathology; (U) Calcium metabolism; (U) Hypocalcemia; (U) Renin; (U) Angiotensin; (U) Humans; (U) Dogs									
31. TECHNICAL OBJECTIVE, 32. APPROACH, 33. PROGRESS (Provide individual paragraphs identified by number. Provide text of each with Security Classification Code)									
23. (U) To study the feasibility of using lyophilized umbilical veins for creation of A-V fistula in experimental animals as a prototype for vascular access in hemodialysis patients. To determine the cause of immune deficiency in patients with renal failure. To assess the pathophysiology of acute renal failure based on histopathic changes. To evaluate the role of parathyroid hormone in the calcium metabolism of burned soldiers. To perfect a chronic awake intact canine model for renal physiologic studies.									
24. (U) Lyophilized human umbilical veins are to be used to create A-V fistula in the neck of dogs. A battery of immunologic testing is to be done on patients with renal failure and correlated with nutritional status. Light microscopy, scanning electron microscopy and transmission electron microscopy is to be correlated with clinical course of patients with renal failure. Serial determinations of calcium, phosphorus, cyclic AMP and PTH values in the burn patient are to be determined. Catheters are to be inserted in the renal artery, both renal veins, and both ureters to allow sampling in an effort to assess the role of calcium in the modulation of the renin-angiotensin system.									
25. (U) 7710 - 7809 Early rupture led to the conclusion that the dog is not an adequate model for the experimental A-V fistulas. A profile of immune deficiencies have been compiled on chronic renal failure patients and compared to serum amino acid levels. Scanning electron microscopy reveals no consistent clue as to the etiology of depressed glomerular filtration in the burn soldier. Remarkable elevation in urinary cyclic AMP suggests that PTH is activated in response to the hypocalcemia seen in the burn soldier. Significant equipment problems remain to be solved in the development of canine awake intact dog model for physiologic studies.									

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PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 69 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN RESEARCH

REPORT TITLE: STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL
FUNCTION CHANGES IN INJURED SOLDIERS-CLINICAL
OPERATION, METABOLIC BRANCH, RENAL SECTION,
FOR TREATMENT OF SOLDIERS WITH RENAL FAILURE

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 January 1977 - 31 December 1977

Investigators:

Harry R. Jacobson, M.D., Major, MC
Richard H. Merrill, M.D., Lieutenant Colonel, MC

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Unclassified

ABSTRACT

PROJECT NO. 3S162774A820-00, MILITARY BURN RESEARCH

REPORT TITLE: STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL
FUNCTION CHANGES IN INJURED SOLDIERS - CLINICAL
OPERATION, METABOLIC BRANCH, RENAL SECTION,
FOR TREATMENT OF SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center
Fort Sam Houston, Texas 78234

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Richard H. Merrill, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288 (R1)

A total of 24 hemodialysis treatments and two peritoneal dialyses were performed on four patients during this reporting interval. A significant contraction in the mission of the Renal Section was made necessary by reduction in personnel. The Third Annual Brooke Annual Nephrology Seminar was held 11-13 April 1977.

Hemodialysis
Peritoneal dialysis
Urinary tract infection

STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL FUNCTION CHANGES
IN INJURED SOLDIERS - CLINICAL OPERATION, METABOLIC BRANCH, RENAL
SECTION FOR TREATMENT OF SOLDIERS WITH RENAL FAILURE

The Renal Section of the ISR includes a Nephrologist and two hemodialysis technicians who also function as research technicians. The primary mission of the Renal Section is to support the Clinical Division of the ISR. This support is in the form of consultation on thermally injured patients who develop either renal insufficiency or disorders of body fluids, electrolytes, or acid base disturbances. Patients who develop renal failure are dialyzed when necessary. In addition to the major mission at ISR, the Renal Section has a secondary mission to support the Nephrology Service of the Brooke Army Medical Center. Patients with renal failure at the main hospital of Brooke Army Medical Center are dialyzed by the ISR hemodialysis team. Further support of the BAMC Nephrology Service is provided by the Chief of the ISR Renal Section who participates in patient care at BAMC.

Nine patients with renal failure were dialyzed by the ISR team. Seven non-thermally injured patients were hemodialyzed a total of thirty-eight times. Two thermally injured patients were dialyzed a total of 2 times with both subsequently dying of burn wound sepsis.

In addition to consultation and hemodialysis the Renal Section has a major research commitment both with respect to clinical and more basic research. Two major clinical projects have been continued. The first is a histological study of kidney tissue obtained immediately post mortem from thermally injured patients who develop renal failure. Scanning electron microscopy is now being utilized. The study intends to answer the question of whether or not morphological changes in the glomerulus are responsible for the renal failure seen in the thermally injured.

The second clinical study involves serial urinalyses in thermally injured patients to detect fungus infection and then to determine whether the site of fungal infection is in the upper or lower urinary tract by the use of immunofluorescent antibodies. The ISR hemodialysis technicians have been trained in the immunofluorescent techniques and to date have completed 97 urinalyses on 33 patients.

In addition to these ongoing clinical studies, plans have been formulated to: 1. Perform a retrospective study on all thermally injured patients admitted to the ISR in 1976 with the intentions of identifying all patients who developed renal failure, and compare their course with patients who maintained normal renal function. From this date it is hoped that predictive and risk factors may be obtained. 2. Perform a

prospective study on thermally injured patients first to describe the changes in renal function that occur with burn injury and resuscitation (renal blood flow, glomerular filtration, osmolar clearance, fractional excretion of various solutes), and second to describe the pathophysiology when these patients developed impaired renal function.

Finally, USAISR Nephrologists coordinated the Third Annual Brooke Army Medical Center Nephrology Seminar, a three-day event devoted to kidney physiology and clinical disorders of renal functions. Guest speakers from all over the United States participated and the meeting was sanctioned by the American College of Physicians as a post graduate education course. The Fourth Annual Nephrology Seminar entitled, "Hypertension Update" will be held on 11, 12, and 13 October 1978.

PRESENTATIONS AND/OR PRESENTATIONS

None

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN RESEARCH

REPORT TITLE: STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL
FUNCTION CHANGES IN INJURED SOLDIERS-IMMUNE
DEFICIENCY IN DIALYSIS PATIENTS: A STUDY OF ACUTE
RENAL INSUFFICIENCY AND RENAL FUNCTION CHANGES IN
INJURED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigator:

Richard H. Merrill, M.D., Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288 (R1)

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ABSTRACT

PROJECT NO. 3S162774A820-00, MILITARY BURN RESEARCH

REPORT TITLE: STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL
FUNCTION CHANGES IN INJURED SOLDIERS - IMMUNE DEFICIENCY
IN DIALYSIS PATIENTS: A STUDY OF ACUTE RENAL INSUFFICIENCY
AND RENAL FUNCTION CHANGES IN INJURED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

Investigator: Richard H. Merrill, M.D., LTC, MC

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It has been assumed that the depressed cellular immunity in patients with chronic renal failure is due to some "uremic toxin". However, there are many groups of patients and clinical situations characterized by a depression of cell mediated immunity but having normal renal function; among them protein-calorie malnutrition and phosphate depletion. If a uremic toxin is responsible, rather than a deficiency state, the presence of depressed cellular immunity should correlate with the degree of renal insufficiency.

Forty-three patients with end-stage renal disease on chronic hemodialysis and fifteen patients with varying degrees of renal failure on conservative management were studied. Neutrophil chemotaxis was assayed and T and B lymphocytes were enumerated. B lymphocytes were similar in both groups and not different from normals, while T cells were depressed in the patients on dialysis. There was no correlation between serum creatinine and chemotaxis in the non-dialysis group, but there was a tendency for chemotaxis to become more abnormal as length of time on chronic dialysis increased. Complement and immunoglobulins were normal in both groups.

This data indicates that the depressed cellular immunity seen in patients with end-stage renal disease may be due to protein-calorie malnutrition or other deficiency rather than a uremic toxin.

Lymphocytes
Chemotaxis
Cellular immunity

PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN RESEARCH

REPORT TITLE: STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL FUNCTION
CHANGES IN INJURED SOLDIERS -- RENAL FUNCTION IN THE
BURNED SOLDIER. I. ELECTRON MICROSCOPY

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 October 1977 - 30 September 1978

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ABSTRACT

PROJECT NO. 3S162774A820-00, MILITARY BURN RESEARCH

REPORT TITLE: STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL FUNCTION
CHANGES IN INJURED SOLDIERS -- RENAL FUNCTION IN THE
BURNED SOLDIER. I. ELECTRON MICROSCOPY

US Army Institute of Surgical Research, Brooke Army Medical Center,
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Period covered in this report: 1 October 1977 - 30 September 1978

Investigators: Paulette C. Langlinais, MS
W. Duke Myers, LTC, MC, FACP
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Reports Control Symbol MEDDH-288(R1)

Previous electron microscopic (SEM) studies have suggested morphological changes occur in the renal glomerulus of animals in experimental acute renal failure (ARF). No studies have been done on the human glomerulus in ARF. Sixteen thermally injured patients were studied by SEM and the observations were correlated with the light microscopy and clinical data. The findings indicate that there are no specific morphological alterations associated with ARF in the human.

Acute renal failure
Scanning electron microscopy
Renal glomerulus

STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL FUNCTION CHANGES
IN INJURED SOLDIERS -- RENAL FUNCTION IN THE BURNED
SOLDIER. I. ELECTRON MICROSCOPY

Descriptive morphology by scanning electron microscopy (SEM) of the glomerulus in acute renal failure (ARF) has been limited to the study of animal tissue in experimentally induced ARF utilizing either ischemia (1, 2) or one of several nephrotoxic agents (2-7). In spite of these studies, the etiology of ARF continues to be an enigma. Although the need for corresponding definitive studies in man has been stressed, human tissue is not readily available for study. The thermally injured patient population at our Institute provides a relatively high frequency of ARF. Previously, we studied a group of 25 patients by light, transmission electron and immunofluorescent microscopy (8). We found no evidence that fibrin or immune complex deposition or basement membrane alteration was responsible for ARF. However, because of recently observed glomerular surface alterations by scanning electron microscopy in experimental ARF, a new study was designed to examine patients with ARF, as well as patients with graded renal impairment, using the scanning electron microscope.

-
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 8. Myers WD, Merrill RH, Langlinais PC: Studies of acute renal insufficiency and renal function changes in injured soldiers. Annual Research Progress Report, USAISR, BAMC, Ft Sam Houston, TX, 1 Oct 76 - 30 Sep 77, p. 336.

MATERIALS AND METHODS

A large number of patients admitted to the Institute of Surgical Research burn unit were studied in a prospective manner. Of these, 16 patients who did not survive their thermal injury were used in the study. Percutaneous needle biopsies were performed within one hour following death. The renal biopsies were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3, at 4° C for 24 hours. Fixed specimens were washed overnight in buffer, dehydrated in graded ethanol/water solutions to absolute ethanol, then through graded ethanol/Freon 113 solutions to absolute Freon 113. The specimens were dried by the critical point method in a Bomar SPC 900 utilizing Freon-13. Dried specimens were coated with gold/palladium in a Hummer D C Sputtering system, and examined in an ETEC Autoscan at either 10 or 20 KV.

Each specimen was evaluated for morphological changes by SEM without prior knowledge of their clinical status, or of the light microscopic diagnosis. The entire biopsy was systematically examined to insure that every glomerulus present was surveyed to determine both the number of glomeruli present in each biopsy, as well as the extent of involvement when morphological changes were present. The glomeruli counted averaged 9 per biopsy with a range of as few as 4 and as many as 26. The morphological alterations were judged by 3 criteria: (1) type of change, (2) severity of change and (3) distribution, i.e., focal or diffuse. To facilitate evaluating the severity of change, an arbitrary scale of 0-10 was used in rating these criteria.

Light microscopy was done on routinely prepared formalin fixed tissue taken at the time of post mortem examination. The paraffin sections were stained with hematoxylin and eosin.

The sixteen patients used in the study were placed into one of two groups, normal versus renal impairment, based on the results of laboratory tests done on the last day that complete values were available. This did not always correspond to the day of death. Clinical data used included electrolytes, blood urea nitrogen (BUN), and creatinine plus other values obtained by routine chemical profile. The urinary values were obtained from spot urines. In addition to the laboratory results, data on the administration of nephrotoxic antibiotics was also considered.

OBSERVATIONS

The morphological results of the study and clinical data are summarized in Table I. Three major morphological changes were observed by SEM. The most prominent alteration of normal glomerular architecture was fusion, a complete loss of interdigitation of the foot processes resulting in a sheet-like appearance of the podocyte cytoplasm. The second change was flattening of the primary and secondary foot processes of the podocytes giving them a thin, wrinkled appearance, but with no loss of interdigitation of the pedicels. The third, and least prominent change,

TABLE 1 - MORPHOLOGICAL AND CLINICAL DATA

Patient Code	Age	Sex	DPB	Scanning Electron Microscopy				Pathology	Clinical Status	Nephrotoxic Antibiotics
				Fusion	Flattening	Cap Const	Other			
A	26	M	14		+2 f		+1 retraction	Normal	R1	Cl
B	46	M	11		+4 f	+1 f		Cortical infarct	N	Cl
C	27	M	1				+5 d erosion	ATN	N	None
D	22	M	25		+5 d	+5 d		Early ATN	N	Cl
E	79	F	7	+4 d	+4 d		+5 retraction	Medullary fibromas	N	Cl
F	50	M	10		+2 f			Interstitial nephritis	N	Cl
G	21	M	15	+8 d			1 fibrosed glomerulus	f glomerular fibrosis	R1	Cl
H	21	M	8		+2 f	+1 f		Normal	R1	Cl
I	6	F	20	+3 d	+3 d			Normal	N	Cl
J	59	M	36	+8 d		+4 f		Early ATN	ARF	Cl
K	25	M	9	+8 f	+4 f	+1 d	+1 retraction	Normal	R1	Cl
L	2	F	7					Normal	ARF	None
M	74	F	35	+2 f			Numerous microvilli	d parenchymal fibrosis	ARF	Cl
N	22	M	3	+5 d			Cell body shrivelling	ATN	R1	Ge
O	44	M	40	+9 f			Cell body shrivelling	Normal	ARF	Cl
P	33	M	28	+2 d			Numerous microvilli	ATN	R1	Cl, Am, Ge

Key:

d - diffuse

f - focal

DPB - days post burn

Cap Const - capillary constriction

ATN - acute tubular necrosis

ARF - acute renal failure

N - normal

R1 - renal impairment

Cl - Colistin

Ge - Gentamicin

Am - Amphotericin

was capillary constriction in which the capillary loops of the glomerulus appeared narrow and sinuous. Some of the other isolated observed changes were retraction of foot processes exposing basement membrane, erosion of the podocyte cell body and primary processes, swelling of the cell bodies, shriveling of the cell bodies, and numerous microvilli appearing on the cell body and foot processes. The observed alterations were considered to be focal if they were present in small areas of a glomerulus even though most of all of the glomeruli were affected. The alterations were considered diffuse if they were found over large areas of the glomerulus and nearly all glomeruli of the biopsy showed some change. Only one patient in the study, L, showed no change by SEM. The remaining fifteen patients had one or more morphological alterations.

Light microscopy showed only one patient with glomerular changes. Of the remaining fifteen patients, five were essentially normal; five had acute tubular necrosis; and the remaining five had a variety of renal changes including medullary fibromas, cortical infarct, focal glomerular fibrosis, and diffuse parenchymal fibrosis. As indicated by SEM, patient L showed no renal alterations.

Fourteen patients received at least one antibiotic considered to be nephrotoxic, and one of the patients, P, received three nephrotoxic antibiotics. Two of the patients, C and L, received no nephrotoxic antibiotics. The antibiotics identified as nephrotoxic were Colistin, Gentamicin and Amphotericin.

In evaluating the clinical laboratory findings, six patients were within normal limits while the remaining ten patients had test results indicating renal impairment. For the purposes of the study, four of the patients with a serum creatinine of more than 1.5 and oliguria were categorized as having acute renal failure. The remaining six patients had elevated serum creatinine but adequate urine output and were considered to have renal impairment but not clinical acute renal failure. This distinction was made to facilitate a comparison of the SEM alterations with the clinical status of the patient.

In reviewing all of the data presented in Table I, it is obvious that the architectural alterations observed by SEM do not correlate well with either the clinical status of the patient or the light microscopic findings. Patient L had no changes by either SEM or light microscopy but was one of the four patients with acute renal failure by clinical status. Patient K had severe changes by SEM, some renal impairment clinically, but no changes by light microscopy. Patient O had severe changes by SEM, acute renal failure clinically, and normal light microscopy. The nephrotoxic antibiotics appeared to have no correlative value as the one patient, P, that received all four antibiotics had minimal SEM changes and renal impairment clinically. Patient C received none of the antibiotics, had no clinical changes, but had moderate SEM alterations. Patient L had no antibiotics, normal SEM, and clinical acute renal failure.

The results of the observations in this study indicate that morphological alterations present in biopsies viewed by SEM do not correlate directly with the clinical status of the patients. Not only is there no direct correlation, but the type of morphological alteration present is not consistent. It appears that the most frequent and severe change is fusion of the pedicels, but it is not present in every patient with acute renal failure and may even be present in patients with normal clinical and light microscopic evaluations.

DISCUSSION

The present study was undertaken to evaluate the glomerular architectural changes accompanying renal impairment and ARF in humans by SEM. The past several years has produced numerous SEM studies of the renal glomerulus in animal models of ARF, but no observations on the human glomerulus in ARF have appeared. Andrews (9) and Arakawa (10) have studied the normal human glomerulus by SEM providing a basis of comparison.

All of the variety of morphological changes observed by SEM in animals under experimental conditions and described in the literature are present in our human patients (1-6, 11-13). The most frequently occurring change was the loss of definition of the pedicels resulting in sheet-like appearance of the podocyte cytoplasm. We have used the word fusion to describe this architectural change without being able to demonstrate that a fusion of pedicels actually occurs. The retraction and disappearance of the pedicels that we observed have been described in human patients with glomerulonephritis (14). From the variety of morphological changes observed in our patients that compare to the equally numerous changes described in a variety of experimental setting and human disease, two significant findings are evident. First, ARF in humans does not have a

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concomitant morphological change in the renal glomerulus. Although many changes are present in our patients with renal impairment and ARF, no specific change can be identified and in some cases no change of any type is present. Second, morphological changes in the renal glomerulus of humans appear to be non-specific. We are not able, at this time, to discern a pattern of change in a specific clinical condition. This finding is supported by the results of our evaluation of the effects of nephrotoxic antibiotics. Although some morphological change by transmission electron microscopy has been attributed to gentamicin (15), no such changes are evident by SEM.

In conclusion, the present SEM study supports the findings of our previous study on humans (8) that ARF cannot be associated with a morphological change in the renal glomerulus. It does not rule out the possibility that at some stage of ARF there is an alteration in the structure of the glomerulus, but the actual clinical condition of ARF cannot be explained on the basis of morphological changes in the glomerulus.

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PRESENTATIONS AND/OR PUBLICATIONS: None.

TERMINATION

PROJECT NO. 3S162774A820-00-MILITARY BURN RESEARCH

REPORT TITLE: STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL
FUNCTION CHANGES IN INJURED SOLDIERS-EVALUATION
OF CALCIUM METABOLISM IN BURNED TROOPS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigator

Richard H. Merrill, M.D., Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288 (RI)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S762774A820-00-MILITARY BURN TECHNOLOGY

REPORT TITLE: STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL
FUNCTION CHANGES IN INJURED SOLDIERS-EVALUATION
OF CALCIUM METABOLISM IN BURNED TROOPS

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam
Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

Investigator: Richard H. Merrill, M.D., LTC, MC

Reports Control Symbol MEDDH-288 (RI)

To elucidate the mechanism for the hypocalcemia seen early in the post-injury period of patients suffering thermal injury, nineteen patients were studied in detail. Total serum calcium was low in all patients on the first post-burn day, and was low in the majority even when corrected for serum albumin. Serum ionized calciums were obtained on a number of patients but did not correlate well with the total serum calcium. The serum phosphorus was initially depressed in all patients, but was restored to normal in nearly all patients by the seventh post-burn day. This occurred whether or not the patients were given an oral diet. The urinary excretion of calcium was modestly depressed during the first several days following burn injury, but shortly thereafter were borderline elevated to elevated in nearly all of the patients. Eight patients were started on antacid therapy within the first three days post-burn and eleven patients were not. There was no statistical difference in the serum calcium between these two groups. The occurrence of renal impairment defined as a creatinine clearance below 50 cc per minute at any time during the initial ten days of observation also failed to correlate with the serum calcium. Urinary cyclic AMPs were obtained daily on the patients and were elevated from the onset of their clinical course. When viewed as a group the patients were never in profound negative calcium or phosphate balance whether on intravenous feeding or on an oral diet. With recovery the calcium and phosphorus balance studies revealed an increasingly positive balance. Biopsies obtained from normal skin and from the burn wound in the same patient revealed no striking differences in calcium content.

In summary, the hypocalcemia observed early in the post resuscitation course of the thermally injured patients remains unexplained. Since these patients are in zero to positive calcium balance and the hypocalcemia occurs

early in the burn course it is anticipated that the circulating calcium is sequestered in some pool within the body, and that this pool is not the burn wound. The PTH response to the hypocalcemia appears to be brisk as evidenced by the urinary cyclic AMP measurements, and may be important in the course of these patients since it has been speculated that parathormone may be a neurotoxin in patients with end-stage renal disease. Neither the diet nor antacid therapy nor impaired renal function seems to be related to this initial hypocalcemia.

Humans
Acute Renal Failure
Hypocalcemia

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN RESEARCH

PROJECT TITLE: STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL
FUNCTION CHANGES IN INJURED SOLDIERS - THE EFFECT
OF CALCIUM ON THE RENIN-ANGIOTENSIN SYSTEM-USE
OF AN ANIMAL MODEL OF HYPERTENSION

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

Richard H. Merrill, M.D., Lieutenant Colonel, MC
William D. Myers, M.D., Lieutenant Colonel, MC
Thomas J. Lescher, M.D., Major, MC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S162774A820-00, MILITARY BURN RESEARCH

REPORT TITLE: STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL
FUNCTION CHANGES IN INJURED SOLDIERS - THE EFFECTS
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US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

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Reports Control Symbol MEDDH-288 (R1)

No further work on the effect of calcium on the Renin-Angiotensin System has been accomplished. The protocol was delayed awaiting arrival and modification of new equipment, including catheters, pumps, and dog jackets. The surgical part of the model has been accomplished, involving placement of catheters in both renal veins and one renal artery using bilateral staged flank approaches and bilateral ureteral interposition grafts. All catheters are accessible from the skin, allowing innumerable physiologic and pathophysiologic studies to be completed in the awake intact dog. Equipment problems, however, continue to plague this model. Ill fitting dog jackets have caused significant abrasions on the dog leading to significant morbidity. The switch from PVC tubing to silastic tubing has not proven to be beneficial since the soft silastic tubing collapses when any attempt of aspiration is made. Although the blood is available by gravity drainage, this is not ideal for short collections periods. Further work on the equipment problems in this dog model will be necessary to decide whether it is feasible to continue work in this area or abandon the model.

Dog
Calcium-metabolism
Renin-Angiotensin
Renal Physiology

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				DA FORM 6969		78 10 01		DATE OF REPORT	
1. DATE PREVIOUS	2. KIND OF SUMMARY	3. SUMMARY SET	4. SUMMARY SECURITY	5. REPORTING	6. ADMINISTRATION	7. SPECIFIC DATA	8. CONTRACTOR ACCESS	9. LEVEL OF SUM	10. WORK UNIT
77 10 01	D. CHANGE	U	U	NA	NL	<input type="checkbox"/> YES <input type="checkbox"/> NO	<input type="checkbox"/> YES <input type="checkbox"/> NO	A. TYPE UNIT	
11. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER					
6. PRIMARY	62774A	3S162774A820	00	117					
7. 11111111	61102A	3S161102BS05	00	086					
8. CONTRIBUTING									
12. TITLE (Provide with Security Classification Code)									
(U) The Study of Metabolism and Nutritional Effects of Burn Injury in Soldiers (44)									
13. SCIENTIFIC AND TECHNOLOGICAL AREAS									
003500 Clinical Medicine									
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76 10		Cont		DA		C. In-House			
18. CONTRACT GRANT									
Not Applicable									
19. DATES/EFFECTIVE:		20. EXPIRATION:		21. RESOURCES ESTIMATE		22. PROFESSIONAL MAN YRS		23. FUNDS (in thousands)	
B. NUMBER:		C. TYPE:		D. AMOUNT:		E. CUM. AMT.			
F. KIND OF AWARD:		G. CUM. AMT.		FISCAL YEAR		CURRENT			
				78		10.2		240	
				79		11.5		254	
19. RESPONSIBLE DOD ORGANIZATION									
NAME: US Army Institute of Surgical Research									
ADDRESS: Ft Sam Houston, Texas 78234									
RESPONSIBLE INDIVIDUAL									
NAME: Basil A. Pruitt, Jr., COL, MC									
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21. GENERAL USE									
FOREIGN INTELLIGENCE NOT CONSIDERED									
20. PERFORMING ORGANIZATION									
NAME: US Army Institute of Surgical Research									
Surgical Study Branch									
ADDRESS: Ft Sam Houston, Texas 78234									
PRINCIPAL INVESTIGATOR (Furnish NAME if U.S. Academic Institution)									
NAME: Douglas W. Wilmore, MD									
TELEPHONE: 512-221-4733									
SOCIAL SECURITY ACCOUNT NUMBER:									
ASSOCIATE INVESTIGATORS									
NAME:									
NAME:									
DA									
22. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)									
23. (U) Nitrogen balance; (U) Burn injury; (U) Temperature regulation; (U) Environmental control; (U) Protein metabolism; (U) Hormones; (U) Glucose metabolism; (U) Humans									
24. (U) To identify the etiology and humoral mediators of postinjury hypermetabolism and altered thermoregulation in burned soldiers. To assess the nitrogen sparing of varied alimentary regimens. To define alterations and control of blood flow to the wound and other organs, and to determine the rate of nutrient delivery provided by that flow. To describe the effects of thermal injury on hormone production and on protein, glucose, and fat metabolism.									
25. (U) 7710 - 7809 The major portion of the extra heat produced following injury is the result of metabolic, not thermoregulatory drives, although the patients appear biochemically hypothyroid. Blood flow to an injured extremity is markedly increased, although substantial vasodilator activity can not be found in venous effluence in the animal model. Alanine release for the leg is increased following injury and the rate of flux is burn size related and not altered by the presence of an injury on the leg under study. In the rat model, albumin sequestration into the wound was reduced by hyaluronidase administration.									

*Available to contractors upon originator's approval

DD FORM 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF
BURN INJURY IN SOLDIERS -- INCREASED PERIPHERAL
AMINO ACID RELEASE FOLLOWING BURN INJURY

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

L. Howard Aulick, Ph.D, Major, MSC
Douglas W. Wilmore, MD

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

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US Army Institute of Surgical Research, Brooke Army Medical Center,
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Period covered in this report: 1 October 1977 - 30 September 1978

Investigators: L. Howard Aulick, PhD, Major, MSC
Douglas W. Wilmore, MD

Reports Control Symbol MEDDH-288(R1)

Turnover rates of 10 amino acids were determined in 4 normals and 18 burned patients (mean burn size 41% total body surface) by measuring leg blood flow by venous occlusion plethysmography and arterial (A) and femoral venous (FV) amino acid concentrations. Patient arterial plasma amino acid concentrations were generally low or normal although phenylalanine was elevated. Only alanine demonstrated significant A-FV concentration difference ($-9 \pm 2 \mu\text{M}/100 \text{ ml}$ in patients vs -5 ± 1 in controls, mean \pm S.E.M.). Leg blood flow was $6.26 \pm 0.57 \text{ ml}/100 \text{ ml leg volume} \cdot \text{min}$ in the patients and 2.62 ± 0.57 in controls. While the net peripheral release of the ten amino acids was accelerated following injury, only alanine release was consistently greater in the patients ($0.27 \pm 0.05 \mu\text{M}/100 \text{ ml leg volume} \cdot \text{min}$) compared to controls (0.08 ± 0.02). The increased alanine release from legs of patients was generally related to the extent of total body surface injury and oxygen consumption of the patient, but was unrelated to size of limb burn or leg blood flow. The accelerated rate of alanine release from limbs of burn patients relates to the generalized catabolic effects of injury rather than local inflammatory or metabolic events which may occur in the injured extremity.

Venous occlusion plethysmography
Alanine

INCREASED PERIPHERAL AMINO ACID RELEASE FOLLOWING BURN INJURY

Following injury excretion of urinary nitrogen increases and this loss of body protein is generally related to the extent or severity of the trauma. When Cuthbertson concluded his original studies on post injury protein catabolism, he commented that the nitrogen subsequently lost from the body came from systemic stores rather than arising from damaged tissue at the injury site (6). Others have confirmed this concept, and most evidence suggests that the main site of protein catabolism is skeletal muscles: a conclusion based on the magnitude of nitrogen loss, the clinical evidence of muscle wasting and decreased strength, serial body compositional measurements, and muscle biopsies in humans and carcass analysis in small animals. Recent studies demonstrate increased excretion of 3-methyl-histidine in injured and infected patients (15). This non-metabolized amino acid arises only from muscle, and its excretion rate closely parallels muscle protein breakdown.

In this study the net rate of amino acid exchange across the lower extremities of burn patients was determined and the release of amino acids related both to the systemic effects of injury and to the local presence of a burn on the extremity under study.

MATERIALS AND METHODS

Four normal subjects and eighteen non-infected burn patients were selected for study (Table 1). Patient selection criteria, treatment and initial preparation have been previously described (3). Measurements of skin and rectal temperatures (3), oxygen consumption (19) and leg blood flow (3) were performed in the fasting, quietly resting subjects following a two hour equilibration period in an environmental chamber maintained at 30°C and 40-50% relative humidity.

6. Cuthbertson DP: Observations on disturbance of metabolism produced by injury to the limbs. *Quart J Med* 1:233, 1932.

15. Long CL, Schiller WR, Blakemore WS: Muscle protein catabolism in the septic patient as measured by 3-methylhistidine excretion. *Am J Clin Nutr* 30(8):1349, 1977.

3. Aulick LH, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Influence of the burn wound on peripheral circulation in thermally injured patients. *Am J Physiol* 233:H520, 1977.

19. Wilmore DW, Aulick LH, Mason AD Jr, Pruitt BA Jr: Influences of the burn wound on local and systemic responses to injury. *Ann Surg* 186:444, 1977.

Table 1 **SUBJECT CHARACTERISTICS AND
ESTIMATED LEG AMINO ACID RELEASE**

	CONTROLS	PATIENTS
NUMBER OF SUBJECTS	4	18
NUMBER OF STUDIES	4	20
AGE (YEARS)	29	29
WEIGHT (Kg)	75.5	73.5
% TOTAL BODY SURFACE BURN	—	41 (12-74)
% LEG SURFACE BURN	—	33.5 (0-77.5)
POST BURN DAY STUDIED	—	12 (7-21)
OXYGEN CONSUMPTION (ml/m ² ·min)	126±3	211±11 p<0.001
RECTAL TEMPERATURE (°C)	37.0±0.1	38.4±0.2 p<0.001
¹ LEG BLOOD FLOW (ml/100ml leg·min)	2.62±0.57	6.26±0.57 p<0.001
*ESTIMATED LEG AMINO ACID RELEASE (μM/100ml leg·min)	0.18±0.06	1.00±0.30 p<0.05
LEG ALANINE RELEASE (μM/100ml leg·min)	0.08±0.02	0.27±0.05 p<0.01

* RELEASE = (FEMORAL VEIN - ARTERIAL PLASMA CONC.) X LEG PLASMA FLOW
GROUP MEANS ± S.E.M.

Table 2 **LEG ALANINE RELEASE IN PATIENTS
WITH AND WITHOUT EXTENSIVE LIMB BURN**

	SMALL LEG BURN	LARGE LEG BURN
NUMBER OF SUBJECTS	7	7
AGE (YEARS)	32	30
WEIGHT (Kg)	74.3	73.8
% TOTAL BODY SURFACE BURN	35	37
% LEG SURFACE BURN	7.5	50.5
POST BURN DAY STUDIED	13	13
OXYGEN CONSUMPTION (ml/m ² ·min)	192±12	222±23
RECTAL TEMPERATURE (°C)	38.3±0.4	38.3±0.4
ARTERIAL ALANINE CONCENTRATION (μM/100ml plasma)	34±6	30±2
ARTERIAL-FEMORAL VENOUS CONC. (μM/100 ml plasma)	-15±3	-7±2
LEG BLOOD FLOW (ml/100ml leg·min)	3.58±0.30	7.83±0.43 p<0.01
LEG ALANINE RELEASE (μM/100ml leg·min)	0.28±0.07	0.29±0.09

ARTERIAL PLASMA CONCENTRATION

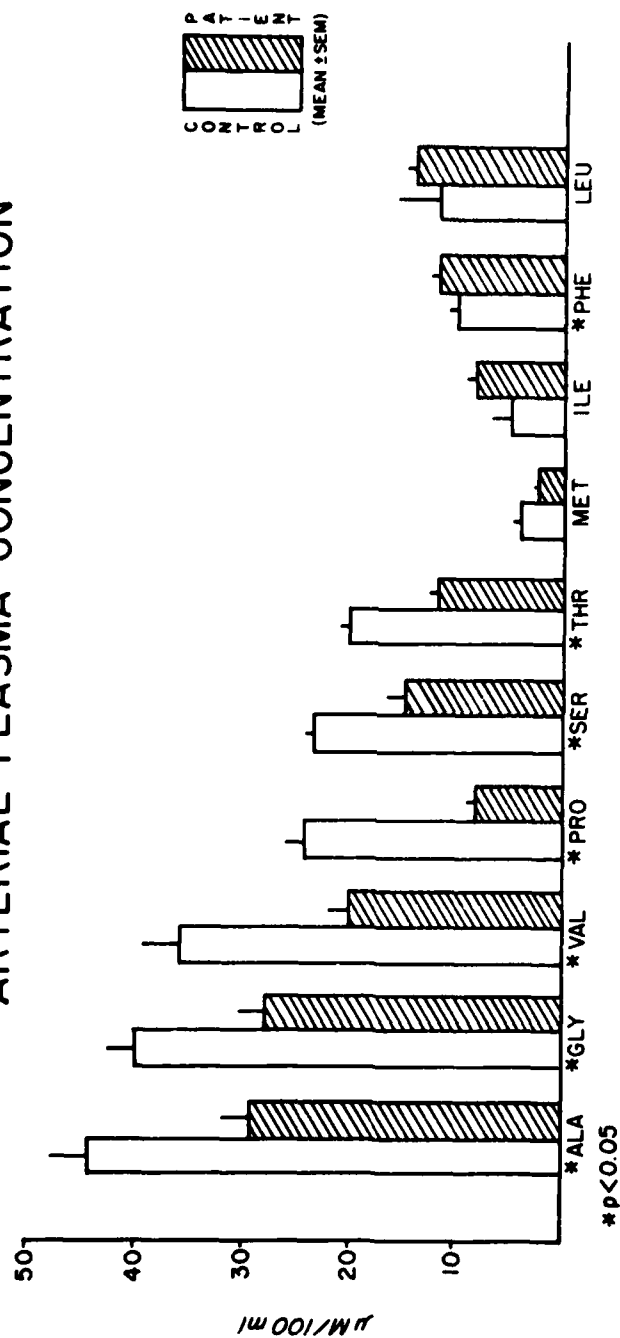


Figure 1

ARTERIAL-FEMORAL VENOUS DIFFERENCE

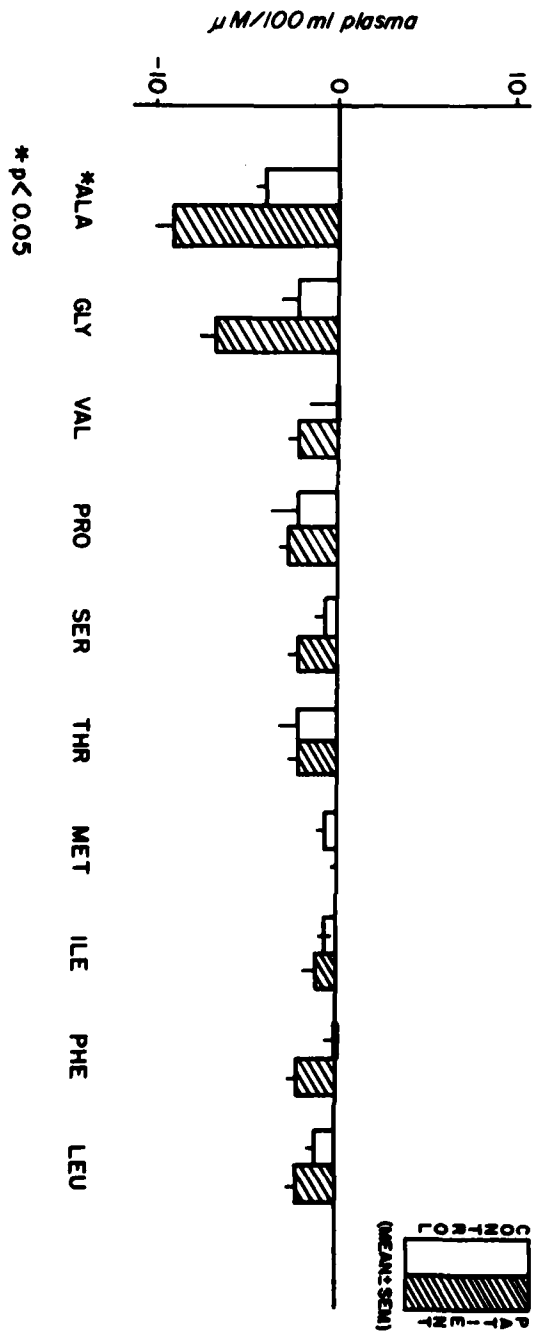


Figure 2

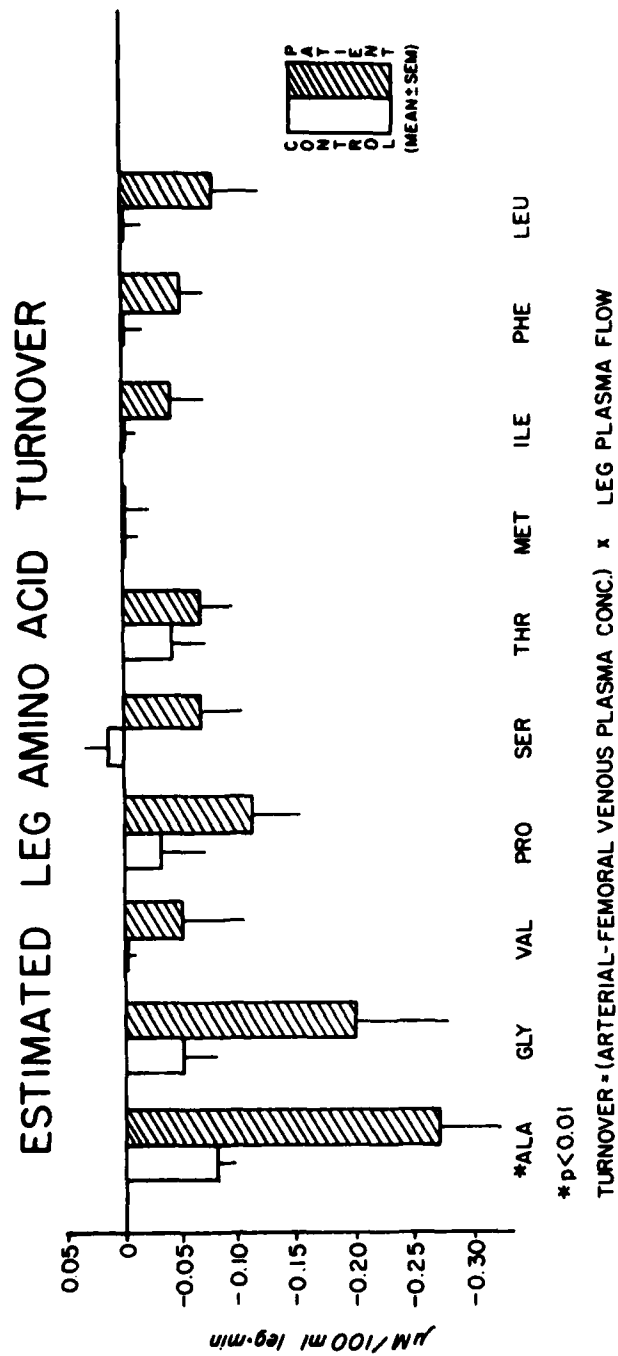
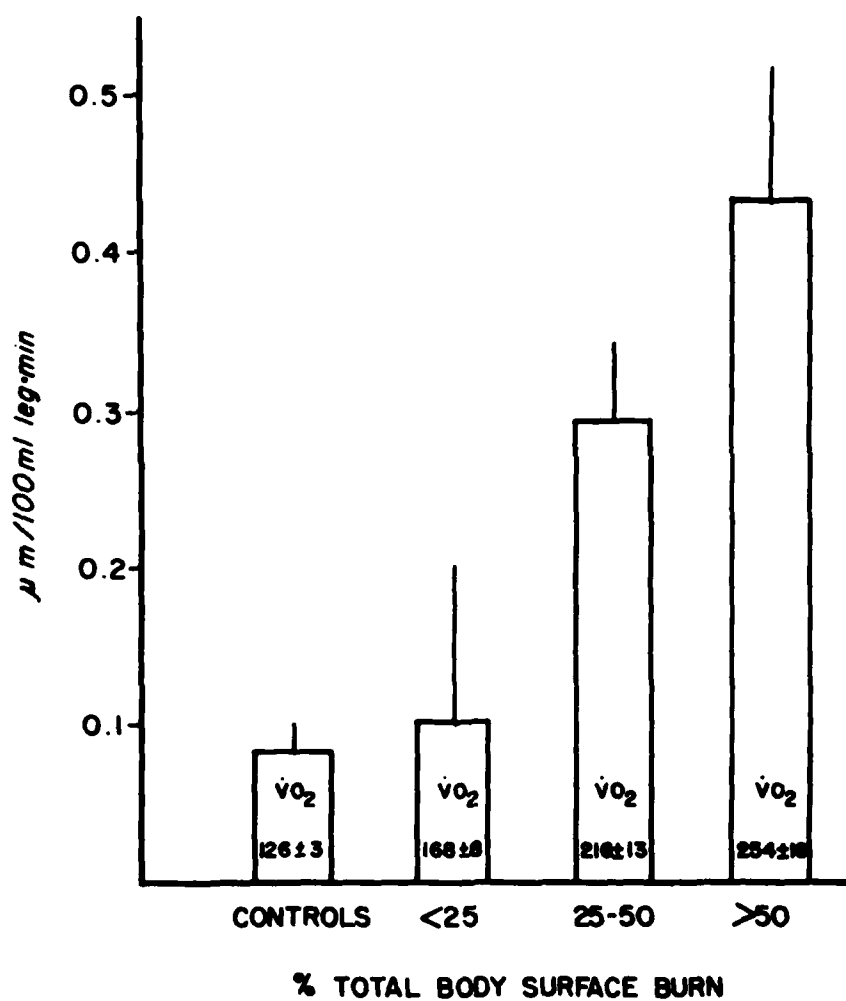


Figure 3

EFFECT OF BURN SIZE ON LEG ALANINE RELEASE



$\dot{V}O_2$ in ml/m²·min (MEAN±SEM)

Figure 4

Following these measurements, blood was simultaneously drawn from the femoral vein of the leg under study and from a previously cannulated, peripheral artery in another limb for determination of arterial and venous plasma amino acid concentrations and hematocrit. Blood samples were immediately centrifugated and the plasma separated and frozen. Amino acid analysis was performed by standard chromatographic techniques after the plasma was deproteinized with sulfosalicylic acid.

Amino acid release from the left leg was calculated as the product of the leg plasma flow [leg blood flow \times (1-HCT/100)] and the arterial-venous plasma concentration difference. Ten plasma amino acids were evaluated.

RESULTS

Arterial plasma concentrations were significantly lower in patients for alanine, glycine, proline, valine, serine and threonine, while leucine, isoleucine and methionine levels were normal and phenylalanine concentration above normal (Fig 1). Only alanine demonstrated significant arterial-femoral vein concentration differences, with the controls averaging $-5 \pm 1 \mu\text{M}/100 \text{ ml}$ and the burn patients -9 ± 2 (mean \pm S.E.M., Fig 2). Leg blood flow averaged $2.62 \pm 0.57 \text{ ml}/100 \text{ ml leg volume} \cdot \text{minute}$ in the controls and 6.26 ± 0.57 in the patients; the mean hematocrit was 39 in the controls and 35 in the patients. Thus, the rate of peripheral alanine release was markedly elevated in the burn patients ($0.27 \pm 0.05 \mu\text{M}/100 \text{ ml leg volume} \cdot \text{min}$) compared to the controls (0.08 ± 0.02 , $p < 0.01$). Although the peripheral release of any other single amino acid was not significantly above control levels (Fig 3), the composite, net release of all ten amino acids was five fold greater in the patients -- $1.00 \pm 0.30 \mu\text{M}/100 \text{ ml leg vol} \cdot \text{min}$ vs 0.18 ± 0.06 for controls ($p < 0.05$).

The increase in alanine release from the resting leg was generally related to the extent of total body surface injury and to the oxygen consumption of the patient (Fig 4). However, alanine release was unrelated to leg blood flow ($r^2=0.002$) or the extent of burn on the extremity ($r^2=0.15$).

To standardize the systemic influences on peripheral events and thereby identify the effect of a local injury on alanine release, patients were matched for age, total body surface injury and associated systemic responses to injury (Table 2). One group had minimal or no leg burns (mean=7.5% leg surface) while the other had major leg burns (50.5%). The systemic responses to injury were comparable in both groups as reflected by similar oxygen consumption and rectal

temperature. Arterial alanine concentrations were also comparable. Leg blood flow was increased significantly by the local presence of a wound. In spite of this dramatic increase in extremity blood flow, leg alanine release was comparable in both groups of patients.

DISCUSSION

Fundamental research in amino acid metabolism in the past decade has defined the intermediary steps in protein catabolism and described amino acid exchange between various organs. In studies of post absorptive individuals the increased peripheral amino acid release is, in general, quantitatively matched by the net uptake of amino acids across the splanchnic bed (8). This carcass-to-viscera exchange of amino acids increases with starvation (9) and diabetes (17). Similar amino acid transfer has also been observed in exercising normals, with the rate of amino acid exchange generally related to the intensity of the exercise (1).

It is well known that amino acids are transported by the blood both in the plasma and red blood cells (2,7). In particular, glutamine is transported by the erythrocyte, especially following a protein meal. Chiasson and associates have provided evidence that 92% of alanine exchange across the splanchnic bed is derived from plasma in individuals studied after an overnight fast (5), although muscle exchange to plasma may be slightly lower (8). Thus, plasma

8. Felig P: Amino acid metabolism in man. *Annu Rev Biochem* 44: 933, 1975.

9. Felig P, Owen OE, Wahren J, Cahill GG: Amino acid metabolism during prolonged starvation. *J Clin Invest* 48:584, 1969.

17. Wahren J, Felig P, Cerasi E, Luft R: Splanchnic and peripheral glucose and amino acid metabolism in diabetes mellitus. *J Clin Invest* 51:1870, 1972.

1. Ahlborg G, Felig P, Hagenfeldt L, Hendler R, Wahren J: Substrate turnover during prolonged exercise in man. *J Clin Invest* 53: 1080, 1974.

2. Aoki TT, Muller WA, Brennan MF, Cahill GF Jr: Blood cell and plasma amino acid levels across forearm muscle during a protein meal. *Diabetes* 22:768, 1973.

7. Elwyn DH, Launder WJ, Parikh HC, Wise EM: Roles of plasma and erythrocytes in interorgan transport of amino acids in dogs. *Am J Physiol* 222:1333, 1972.

5. Chiasson JL, Liljenquist JE, Sinclair-Smith BC et al: Gluconeogenesis from alanine in normal postabsorptive man. Intrahepatic stimulatory effect of glucagon. *Diabetes* 24:574, 1975.

concentrations are utilized by most workers in this field under steady state conditions, although plasma measurements alone will underestimate overall interorgan amino acid exchange. However, as pointed out by Felig, "the direction of transfer and the relative contributions of individual amino acids are not obscured by measurements restricted to the plasma compartment."

The plasma concentrations of various amino acids have been previously measured in burn patients and found, for the most part, to be below normal (21). The major exception is phenylalanine which is elevated (13,21); increase in this aromatic amino acid concentration occurs following a wide variety of critical illnesses (10,18). Previous studies demonstrate that the total body turnover of phenylalanine is markedly elevated following thermal injury and is accelerated even more when the burn patient becomes infected (13).

Studies in post absorptive normals demonstrate that a net release of almost all amino acids occurs from skeletal muscle (8,14). The reported arteriovenous differences across skeletal muscle of the forearm or leg are widely varied. Alanine A-V differences in normal, post absorptive subjects, for example, ranged from 4.4-11.8 $\mu\text{M}/100\text{ ml}$ (14). Although these disparities may reflect analytical differences, they may in fact follow physiological variations in extremity blood flow. Extremity A-V differences of amino acids from our four controls fell within the range of normals previously reported. While A-V differences in the burn patients were also

21. Wilmore DW, Mason AD Jr, Pruitt BA Jr: Impaired glucose flow in burned patients with gram-negative sepsis. *Surg Gynecol Obstet* 143:720, 1976.

13. Herndon DN, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Abnormalities of phenylalanine and tyrosine kinetics: significance in septic and nonseptic burned patients. *Arch Surg* 113:133, 1978.

10. Fisher JE, Rosen HM, Ebeid AM, James JH, et al: The effect of normalization of plasma amino acids on hepatic encephalopathy in man. *Surgery* 80:77, 1976.

18. Wannemacher RW Jr, Klainer AS, Dinterman RE, Beisel WR: The significance and mechanism of an increased serum phenylalanine-tyrosine ratio during infection. *Am J Clin Nutr* 29:997, 1976.

8. Felig P: Amino acid metabolism in man. *Annu Rev Biochem* 44:933, 1975.

14. Liljenquist JE, Lacy WW, Chiasson J-L, Raninowitz D: Regulation of alanine and branched chain amino acid metabolism in intact man, in *Clinical Nutrition Update: Amino Acids*, Chicago, 1977, American Medical Association, pp 22-33.

within this range, extremity blood flow in these patients ranged from normal to 2-3 times normal depending on the size of limb burn (3). As blood concentrations depend on rates of local metabolism and flow, it is important to note that in the patients only the A-V difference for alanine was significantly elevated above controls. Previous studies have partitioned leg blood flow in burn patients and demonstrated that muscle blood flow is normal and that most, if not all, of the extra blood flow is directed to the surface wound (4). Of the 10 amino acids studied, only alanine was released in sufficient quantities to override, even in the injured legs, the dilutional effects of increased limb flow. Therefore, peripheral substrate turnover cannot be adequately assessed by regional A-V differences alone; these measurements must be complimented by local blood flow determinations.

When plasma amino acid A-V concentration difference is multiplied by extremity plasma flow, burn patients demonstrate an increased peripheral release of amino acids. Using alanine as a catabolic marker, the increased release was related to the systemic responses to injury and the rate of alanine release was generally related to per cent total body surface burn and oxygen consumption of the patients. Alanine release was not related to the presence of local injury on the extremity, thus supporting the initial suggestion of Cuthbertson that protein breakdown occurs as a generalized or total body response to injury, and is not the result of nitrogen released from the area of injury (6).

It has been proposed that alanine serves as a major "carrier" of nitrogen from skeletal muscle to liver, and hepatic turnover studies in normals demonstrate that alanine accounts for approximately 50% of the total hepatic amino acid uptake (8). Utilizing leg volume measurements taken on our subjects, a calculation of whole leg alanine release can be made and total skeletal muscle alanine release estimated (Table 3). Assuming that all alanine is released

3. Aulick LH, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Influence of the burn wound on peripheral circulation in thermally injured patients. *Am J Physiol* 233:H520, 1977.

4. Aulick LH, Wilmore DW, Mason AD Jr: Muscle blood flow in burn patients. *The Physiologist* 20:3, 1977.

6. Cuthbertson DP: Observations on disturbance of metabolism produced by injury to the limbs. *Quart J Med* 1:233, 1932.

8. Felig P: Amino acid metabolism in man. *Annu Rev Biochem* 44: 933, 1975.

from muscle and that one leg contains 1/6th of the total muscle mass, control values yield an alanine skeletal muscle release of 48-58 $\mu\text{M}/\text{min}$, rates comparable to the 52 $\mu\text{M}/\text{min}$ reported by others (14). Similar calculations in the burn patients demonstrate a 3-4 fold increase in total alanine release from resting skeletal muscles. Assuming that all the alanine was converted to glucose, this single gluconeogenic amino acid precursor would account for approximately 0.14 M (25g) of glucose/day. If alanine represents only 50% of the amino acids converted to glucose by the liver, gluconeogenesis of peripheral amino acids would produce close to 0.28 M (50g) glucose each day. Estimates of hepatic glucose production in burn patients suggest that it is 2-3 times normal levels following burn injury (16,20). Thus the 0.14-0.28 M (25-50g) glucose which could arise from amino acids is only a small fraction of the 2.22-3.33 M (400-600g) glucose produced each day in these patients. This estimate emphasizes the relatively small contribution made by amino acids to the total hepatic production of glucose;

Table 3. Estimated Peripheral Alanine Release

Net Release	Controls	Burn Patients
100ml Leg Volume ($\mu\text{M}/100\text{ml leg}\cdot\text{min}$)	0.08	0.27
Whole Leg ($\mu\text{M}/\text{min}$)	8-10	27-32
Total Skeletal Muscle ($\mu\text{M}/\text{min}$)	48-58	162-192

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similar calculations confirm that alanine contributes no more than 10% of the glucose formed after three days of starvation (11). Conversely, this estimate emphasizes the important role of other three carbon precursors (lactate, pyruvate and glycerol) in supporting gluconeogenesis in injured man.

Since alanine represents no more than 10 per cent of skeletal muscle protein yet accounts for the major portion of the peripheral amino acid release, *de novo* synthesis of alanine must occur. Currently, two metabolic pathways have been suggested for intracellular alanine synthesis: a proposed glucose-to-alanine cycle (8) and/or protein degradation and transamination (12). Previous studies have demonstrated that glucose uptake across unburned extremities of burn patients is very low (19). Because the increased alanine release occurs in the face of limited glucose consumption, the proposed glucose-to-alanine cycle does not appear to be operative in these injured patients. Increased peripheral alanine release, reflecting skeletal muscle protein degradation, is consistent with the observed muscle wasting, increased excretion of urinary nitrogen and accelerated gluconeogenesis which occurs following thermal injury.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A162774A820-00, MILITARY BURN TECHNOLOGY

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS
OF BURN INJURY IN SOLDIERS - STUDIES OF DISTURBANCE
OF PROTEIN TURNOVER IN BURNED TROOPS - USE OF AN
ANIMAL MODEL

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 October 1977 - 30 September 1978

Investigators:

Wanda L. Brown, MS
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US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
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Groups of male Sprague-Dawley rats weighing 180-200 gm were anesthetized with sodium pentobarbital, the dorsal hair was clipped, and they were subjected to a full thickness scald burn or a sham burn of 20% of body surface. At one hour postburn, rats from each group were given a subcutaneous injection into the wound area of 1 ml of hyaluronidase (150 N.F. units). Other rats from each group received no treatment. The rats were housed in individual cages and were permitted free access to food and water.

Rats from each group were sacrificed at intervals up to 6 days postburn. Under methoxyflurane anesthesia the entire burn or sham wound was excised and immediately weighed. The tissue was minced, homogenized in 9 volumes of 0.1% deoxycholate in 0.15 M NaCl, pH 8.0, and centrifuged (all at 4°C). The supernate was removed and was stored at -20°C until immediately before it was to be analyzed. Albumin content of the plasmas and tissue extracts was determined by radioimmunoassay. Plasma volume was determined by isotope dilution after injection of ¹³¹I-labeled albumin.

The total plasma albumin of the untreated and hyaluronidase-treated burned rats differed significantly only at 3 hours and at 144 hours postburn; that of the hyaluronidase-treated sham rats was significantly lower than that of the untreated sham rats at every time interval (Table 1). The plasma albumin pool sizes of both groups of burned rats were significantly smaller than those of either group of sham burned rats.

The albumin content of the wound area of the hyaluronidase-treated burned rats was greater than that of the untreated burned rats at 3 hours postburn but was smaller after that time (Table 2). The differences between groups were statistically significant. Albumin content in the hyaluronidase-treated sham wound showed a small, but statistically significant, decrease

Table 1. Total Plasma Albumin of Sham and Burned Rats

Hour Post- burn	Untreated (BU)	Burned Hyaluronidase (BHY)	mg Total Plasma Albumin		Hyaluronidase (SHY)	ANOVA Comparisons p =	
			Untreated (SU)	Sham		BU vs BHY	SU vs SHY (BU + BHY) vs (SU + SHY)
1	122.47 ± 2.05* (2)**	-	198.33 ± 11.34 (2)	-	-	-	-
3	139.52 ± 2.12 (4)	167.21 ± 1.44 (3)	231.16 ± 34.91 (2)	-	-	<0.05	-
24	215.31 ± 6.23 (9)	216.32 ± 10.59 (9)	324.76 ± 19.32 (6)	273.12 ± 25.05 (7)	-	NS	<0.001
48	210.79 ± 3.47 (7)	209.58 ± 12.04 (9)	328.39 ± 20.01 (6)	271.27 ± 15.80 (7)	-	NS	<0.001
72	261.62 ± 14.96 (6)	237.36 ± 8.70 (7)	346.13 ± 4.16 (3)	288.33 ± 11.31 (4)	-	NS	<0.001
144	289.13 ± 13.05 (8)	217.31 ± 7.84 (4)	356.06 ± 18.50 (4)	287.58 ± 23.44 (4)	-	<0.01	<0.05

* Mean ± Standard error of the mean,

** (N) Number of rats in group.

Data were Ln transformed for analysis of variance.

Rats were subjected to a scald burn or sham burn of 20% of body surface. Hyaluronidase was injected subcutaneously into the wound area at 1 hour postburn.

Table 2. Total Albumin of Sham and Burn Wound of Rats

Hour Post-burn	Untreated (BU)	mg Total Albumin in Wound Area		Untreated (SU)	Sham Hyaluronidase (SHY)	ANOVA Comparisons $P =$		
		Burned Hyaluronidase (BHY)				BU vs BHY	SU vs SHY	(BU + BHY) vs (SU vs SHY)
1	133.03 \pm 3.14*	-		28.71 \pm 5.15 (2)	-	-	-	-
3	122.18 \pm 6.84 (4)	164.00 \pm 7.32 (3)		26.93 \pm 4.64 (2)	-	<0.05	-	-
24	226.53 \pm 6.46 (9)	130.20 \pm 9.14 (9)		43.06 \pm 4.20 (6)	35.15 \pm 2.39 (7)	<0.001	NS	<0.001
48	212.04 \pm 11.35 (7)	130.86 \pm 5.42 (9)		44.53 \pm 2.54 (6)	36.89 \pm 1.44 (7)	<0.001	<0.05	<0.001
72	207.61 \pm 9.85 (6)	149.52 \pm 6.08 (7)		47.50 \pm 3.00 (3)	33.05 \pm 1.35 (4)	<0.001	<0.001	<0.001
144	265.58 \pm 19.51 (8)	216.29 \pm 2.99 (4)		56.58 \pm 3.39 (4)	35.05 \pm 0.22 (4)	NS	<0.001	<0.001

* Mean \pm standard error of the mean

** (N) Number of rats in group.

Data were Ln transformed for analysis of variance

Rats were subjected to a scald burn or sham burn of 20% of body surface. Hyaluronidase was injected subcutaneously into the wound area at 1 hour postburn.

when compared with that of the untreated sham wound.

¹²⁵I-labeled rat albumin was injected into rats from each group at 24, 48, or 72 hours postburn and the amount which entered the wound area during the following hour was determined. The wound areas of the untreated and hyaluronidase-treated burned rats contained twice as much ¹²⁵I-labeled albumin as did those of the rats in either sham group. Since the total wound albumin did not change significantly during this time, one can assume that the rate of albumin return from the wound was also accelerated in these animals. Transfer rates will be reported later.

ANNUAL PROGRESS REPORT

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GOAT MODEL FOR THE STUDY OF WOUND BLOOD FLOW AND
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Peripheral circulatory and metabolic adjustments to surface injury were monitored in conscious and anesthetized young goats (20-40 kg) by measuring external iliac artery flow bilaterally with Doppler flow probes and arterial-femoral venous blood composition before and for three weeks after removal of the skin from one hindlimb. Over this period, blood flow to the uninjured limb remained unchanged (107 ± 19 ml/min, mean \pm S.E.) while that to the injured leg rose to twice normal levels (186 ± 27 p < 0.01) by the end of one week, associated with the development of granulation tissue. This increase in flow to the injured limb occurred without a significant increase in leg oxygen consumption (2.66 ± 0.3 ml/min, injured vs 1.99 ± 0.25 , uninjured) but was associated with increased glucose uptake (7.76 ± 1.11 mg/min, injured vs 2.70 ± 0.62 , uninjured, p < 0.001) and lactate production (3.56 ± 1.26 mg/min, injured vs 1.10 ± 0.72 , uninjured, p < 0.05). As these changes are comparable to those previously reported in burned patients, (1,4) the goat model appears appropriate for further study of the control of wound blood flow. The goat wound is not vasodilated by local hypoxia, since increasing arterial pO_2 from 92.4 ± 13 mmHg to 455 ± 23 has no effect on hindlimb flow. Whatever local factors may contribute to wound hyperemia, a series of cross perfusion studies failed to demonstrate any circulating factor(s) in the venous blood from the injured limb which dilated other peripheral vascular beds. The results of this study clearly indicate that this model provides an appropriate and effective way to study peripheral circulation and metabolism following thermal injury.

Wound blood flow
Metabolism

THE DEVELOPMENT OF A GOAT MODEL FOR THE STUDY OF WOUND BLOOD FLOW AND METABOLISM

Blood flow to the burn wound is markedly increased during the hypermetabolic-hyperdynamic phase of thermal injury. This increase in surface blood flow occurs when cutaneous vessels in the nearby uninjured skin remain constricted and flow to underlying resting skeletal muscle is normal (1,2). Local and reflex heating studies in burn patients (3) have shown that the neovasculature in the granulating wound has some intrinsic tone but is essentially unresponsive to extrinsic neurogenic drives. This apparent vascular denervation may develop subsequent to either 1) an actual physical disruption of sympathetic vasomotor nerves at the time of injury, or 2) the presence of local inflammatory or metabolic factors which override the influence of extrinsic vasomotor reflexes.

The purpose of this study is to develop a large animal model with a surface wound comparable to that of the burn patient and to determine if such a wound alters peripheral circulation and metabolism in a similar manner to the burn wound. Ultimately, an appropriate model would then be utilized to study anatomical and physiological factors responsible for wound directed, peripheral blood flow.

THE MODEL

Young goats, weighing 20-40 kg, were chosen as the experimental animal. Blood flow was measured in both hindlimbs through the use of Doppler flow probes placed around the external iliac arteries. After surgical implantation of the probes, 7-10 days were allowed for recovery and fixation of the probe to the artery. Leg blood flow was then measured bilaterally for three days in five awake animals, restrained but resting quietly. Once baseline control data was established, the goats were placed under deep anesthesia and the skin excised to fascia from the upper thigh to the hock of one hindlimb. The wound was then covered in a sterile dressing, and bandages changed daily for the remaining three weeks of study. During this time, bilateral hindlimb blood flow measurements were repeated three times each week on awake, resting animals.

1. Aulick LH, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Influence of the burn wound on peripheral circulation in thermally injured patients. *Am J Physiol: Heart and Circulation* 237:901, 1977.

2. Aulick LH, Wilmore DW, Mason AD Jr: Muscle blood flow in burned patients. *The Physiologist* 20(4):3, 1977.

3. Aulick LH, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Depressed reflex vasomotor control of the burn wound. *Annual Research Progress Report, US Army Inst of Surg Res, BAMC, FSHTx, 1 Jul 1976-30 Sept 1977.*

CIRCULATORY AND METABOLIC RESPONSES TO INJURY

Leg blood flow in the injured limbs of these awake animals rose above that to the contralateral, uninjured extremity by the end of the third or fourth day post-excision and remained 80-90 percent greater than that of the control leg for the next two weeks of observation (Figure 1). This increase in blood flow to the injured limb occurred while perfusion of the uninjured leg remained essentially unchanged. Over the three week period of observation, the surface wound developed into a rich, highly vascular bed of granulation tissue. And, with the daily dressing changes, the wound remained noninfected.

Based on this preliminary evidence of increased wound blood flow, a second series of experiments were designed to determine the systemic and peripheral effects of injury. All experiments in this series were performed on anesthetized animals, 9-12 days post-excision. The animals were fasted overnight prior to the study. On the morning of the study, an intravenous injection of Brevital^R sodium (approximately 10 mg/kg) was used for induction of anesthesia. The animals were then intubated, placed supine and maintained under light anesthesia by spontaneously breathing a mixture of penthrane and 100 percent oxygen.

In addition to the bilateral hindlimb flow measurements, systemic blood pressure was monitored directly from the carotid artery throughout all studies. Doppler flow probes were calibrated in situ at the end of each experiment through the use of a Harvard infusion pump.

Systemic and peripheral adjustments to injury were evaluated first by monitoring rectal temperature, blood pressure and simultaneously drawn arterial and bilateral femoral venous blood composition in eleven injured and four uninjured animals. At this point in recovery (9-12 days post-excision) the injured animals were afebrile and normotensive. Arterial blood composition was also normal except for a slight increase in pH and decrease in osmolality (Table 1).

While the systemic effects of injury were relatively insignificant at the time of study, peripheral circulation and glucose metabolism were markedly different in injured and uninjured legs of the same animal. Blood flow to the control limb of the injured animals was 107 ± 19 ml/min (mean \pm S.E.M.) and 186 ± 27 for the excised limbs ($p < 0.01$).

The rate of peripheral substrate turnover was determined in the injured and uninjured limbs by multiplying leg blood flow times the arterial-femoral venous difference. The increase in blood flow to the injured limb occurred without a significant increase in leg oxygen consumption but was associated with a rise in glucose consumption and lactate production (Table 2).

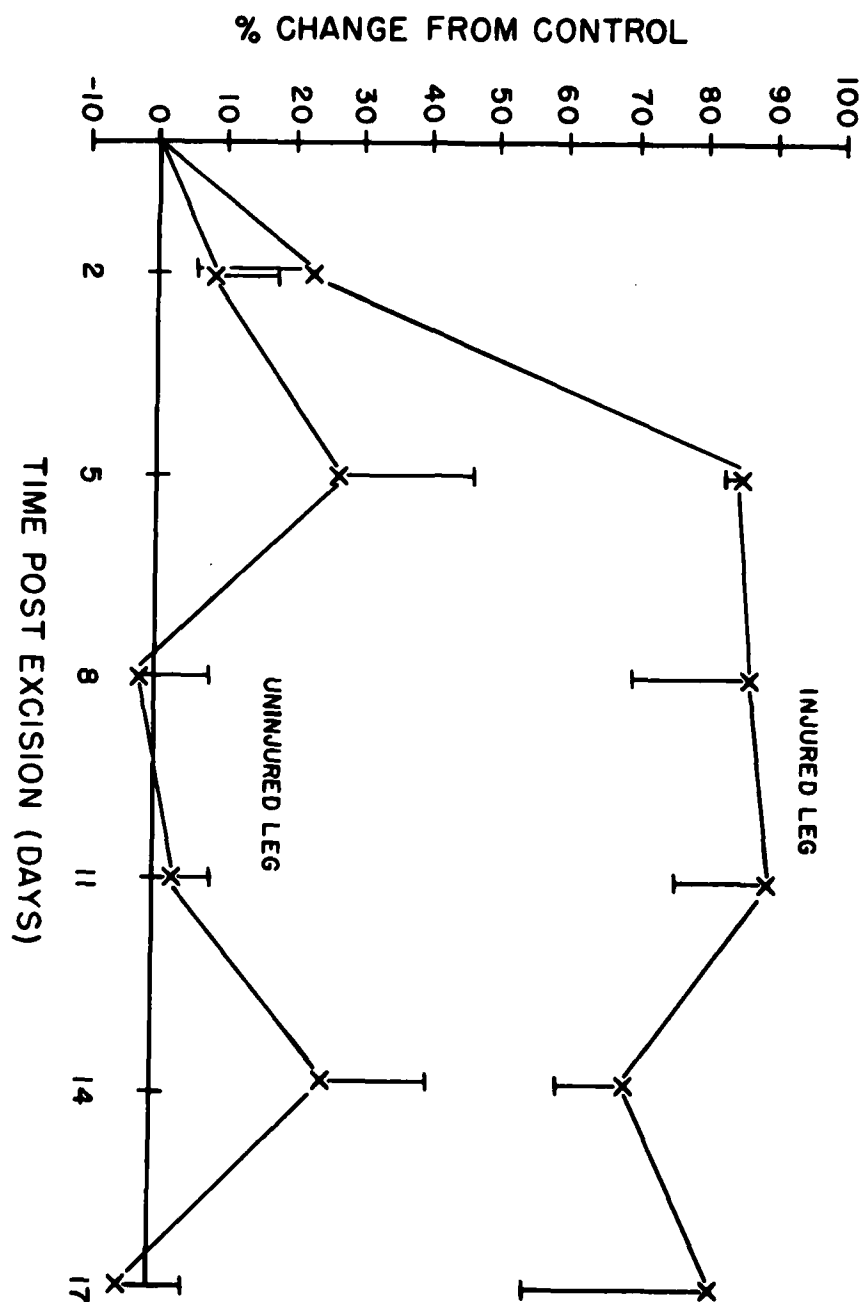


Figure 1

TABLE 1

	<u>Uninjured Animals</u>	<u>Injured Animals</u>
Number	4	11
Weight (kg)	25.0 \pm 1.8	25.0 \pm 1.1
Rectal Temperature ($^{\circ}$ C)	39.2 \pm 0.3	39.2 \pm 0.2
Blood Pressure (mmHg)	125 \pm 2/90 \pm 2	121 \pm 5/86 \pm 5
Arterial		
-pH	7.292 \pm 0.01	7.343 \pm 0.02*
-Hgb (mg %)	9.7 \pm 0.2	9.3 \pm 0.4
-O ₂ content (ml %)	13.0 \pm 0.5	13.1 \pm 0.5
-glucose (mg %)	52 \pm 5	61 \pm 3
-lactate (mg %)	14.6 \pm 3	12.4 \pm 0.9
-pyruvate (mg %)	1.2 \pm 0.1	1.1 \pm 0.04
-sodium (mEq/L)	146 \pm 0.4	144 \pm 0.8
-potassium (mEq/L)	4.1 \pm 0.1	4.6 \pm 0.2
-calcium (mg %)	8.6 \pm 0.5	8.1 \pm 0.1
-osmolality (mosm/L)	301 \pm 1	295 \pm 2*

* p <0.05

TABLE 2

	<u>Injured Goats</u>	
	<u>Uninjured Legs</u>	<u>Injured Legs</u>
Leg Blood Flow (ml/min)	107 ± 19	186 ± 27**
A-FV O ₂ Content (ml/100 ml)	2.3 ± 0.4	1.6 ± 0.2*
Leg Oxygen Consumption (ml/min)	1.99 ± 0.25	2.66 ± 0.3
A-FV Glucose (mg/100 ml)	2.6 ± 0.4	4.4 ± 0.5**
Leg Glucose Consumption (mg/min)	2.70 ± 0.62	7.76 ± 1.11***
A-FV Lactate (mg/100 ml)	-0.9 ± 0.8	-2.0 ± 0.6
Leg Lactate Production (mg/min)	1.10 ± 0.72	3.56 ± 1.26*
A-FV Pyruvate (mg/100 ml)	-0.04 ± 0.05	-0.12 ± 0.13
Leg Pyruvate Production (ng/min)	0.06 ± 0.05	0.20 ± 0.05

*(p <0.05). ** (p <0.01), *** (p <0.001)

Venous blood from the injured limb had higher potassium and lactate concentrations but essentially the same pH, osmolality, sodium, calcium, phosphorous and pyruvate levels as that of the blood draining the contralateral uninjured leg (Table 3).

A series of cross-perfusion studies were performed to determine if differences in venous blood composition have measurable effects on arterial vascular resistance in another peripheral bed. This was accomplished by measuring arterial pressure in the recipient limb while venous blood from either the injured or uninjured limb was pumped at a constant rate into that leg. The recipient or assay limb included a) another uninjured limb of that same animal, b) an intact limb of another uninjured goat, or c) an isolated limb surgically removed from another goat. By maintaining flow constant, variations in vascular resistance with changes in venous blood composition would appear as differences in perfusion pressure ($\text{Resistance} = \text{Pressure} / \text{Flow}$).

In six such cross perfusion studies, vascular resistance in the recipient limb was unaffected by changes in the source of the venous blood perfusate. Using this same experimental approach, we were also unable to demonstrate any change in renal vascular resistance of the same animal, with changes in venous perfusate composition.

The role of oxygen delivery in wound hyperemia was tested in five animals by measuring leg blood flow and arterial oxygen tension after 30 minutes of breathing pure oxygen followed by 30 minutes on room air. Arterial pO_2 dropped from 455 ± 23 to 92.4 ± 13 mmHg (mean \pm S.E.M.) following the shift to room air but blood flow to both injured and uninjured limbs remained essentially unchanged (Table 4).

DISCUSSION

The granulating surface wound, created by surgical excision of the skin from the goat's hindlimb, appears to be an appropriate model for the alterations in peripheral circulation and metabolism observed in burn patients (1,4). The basic similarities are: 1) by the end of one week post-injury, blood flow to injured limbs reach twice control levels associated with the development of a highly vascular wound bed; 2) this increase in peripheral circulation appears to be directed primarily to the surface wound, since blood flow to the uninjured

1. Aulick LH, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Influence of the burn wound on peripheral circulation in thermally injured patients. *Am J Physiol: Heart and Circulation* 237:901, 1977.

4. Wilmore DW, Aulick LH, Mason AD Jr, Pruitt BA Jr: Influence of the burn wound on local and systemic responses to injury. *Ann Surg* 186(4):444, 1977.

TABLE 3

<u>Arterial Concentrations</u>		<u>Venous Concentrations</u>	
		<u>Uninjured Leg</u>	<u>Injured Leg</u>
Sodium (mEq/L)	145 \pm 1	145 \pm 1	145 \pm 1
Potassium (mEq/L)	4.6 \pm 0.2	4.6 \pm 0.2	4.7 \pm 0.2**
Calcium (mg %)	8.1 \pm 0.1	8.3 \pm 0.1	8.3 \pm 0.1
Phosphorous (mEq/L)	6.4 \pm 0.4	6.6 \pm 0.4	6.6 \pm 0.4
Osmolality (mosm/L)	295 \pm 2	296 \pm 2	295 \pm 2
Lactate (mg %)	12.4 \pm 0.9	13.3 \pm 0.8	14.5 \pm 0.9*
pH	7.343 \pm 0.017	7.323 \pm 0.018	7.320 \pm 0.023
Pyruvate (mg %)	1.1 \pm 0.04	1.13 \pm 0.05	1.17 \pm 0.05

*p <0.05

**p <0.01 - Injured different than control by paired T test

extremity does not change; and 3) the increase in injured limb perfusion is not in response to elevated peripheral oxygen consumption but is associated with increased glucose uptake and lactate production.

Through the use of radioactive microspheres Hales (5) has measured leg skin and muscle blood flow in adult sheep. He reported that in these animals standing quietly, leg skin flow averaged 5.0 ml/100 grams · min or 0.56 percent of the measured cardiac output. Since total body blood flow in these sheep (roughly the same size as our goats) was 3.5 liters/min, leg skin flow would be approximately 20 ml/min. Assuming little species variation in surface blood flow, this would indicate that 15-20 percent of the total flow going to the uninjured goat limbs (107 ml/min) was directed to the skin. Peripheral A-V shunts open in anesthetized dogs (6) and conscious sheep in a warm environment (7) suggesting that, since our animals were both anesthetized and under a heating blanket, 20 ml/min should be considered the minimum level of skin circulation in the goat model. Hales (5) calculated that only 12 percent of the cardiac output went to skeletal muscle. Assuming 20 percent of total muscle mass is located in one hindlimb, resting muscle blood flow in sheep would be around 80 ml/min. Recognizing the species variations and differences in experimental design, a rough estimate of the distribution of uninjured goat hindlimb blood flow would allocate 15-20 percent to skin, 75 percent to muscle and the rest to bone, fascia and fat. If most of the extra blood flow to the injured limb is directed to the wound, as suggested by normal flow in the uninjured, control limbs of the goats (Figure 1) and muscle and limb circulatory studies in burn patients (1,2), goat leg skin blood flow would increase from a basal

5. Hales JRS: Radioactive microsphere measurement of cardiac output and regional tissue blood flow in the sheep. *Pflugers Arch* 344:119, 1973.

6. Kaihara S, VanHeerden PD, Migita T, Wagner HN: Measurement of distribution of cardiac output. *J Appl Physiol* 25:696, 1968.

7. Hales JRS: Effects of exposure to hot environments on the regional distribution of blood flow and on cardiorespiratory function in sheep. *Pflugers Arch* 344:133, 1973.

1. Aulick LH, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Influence of the burn wound on peripheral circulation in thermally injured patients. *Am J Physiol: Heart and Circulation* 237:901, 1977.

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level of approximately 15-20 ml/min to 90-110 ml/min. This six to seven fold increase in surface blood flow in the injured leg would mean that 50-60 percent of total limb flow is now directed to the wound and only 30 percent to muscle. Previous estimates in the burn patient with a 50 percent leg surface injury has partitioned limb flow: 80 percent to the surface and 11 percent to resting skeletal muscle. While these calculations do provide a rough estimate of the distribution of peripheral blood flow, they are only general guidelines which must be quantified later.

The observed increase in blood flow to the injured limbs most likely reflects both local anatomical and physiological changes within the wound. The marked increase in capillary density provides the structural basis for wound hyperemia. Numerous inflammatory and metabolic vasodilators have been identified in the burn wound - i.e., prostaglandins, kinins, and lactic acid (4,8-10). The increased release of potassium ions from the injured goats' limbs should now be added to this list and evaluated in man, as these cations are considered to have a potent vasodilator effect in skeletal muscle. While such changes in wound chemistry may have profound local circulatory effects, the results of cross perfusion studies in this animal model indicate that blood draining, injured tissue has little or no apparent effect on the vascular resistance of other peripheral or visceral tissues. This is consistent with previous work which demonstrated no change in flow to uninjured skin and resting skeletal muscles of thermally injured patients.

Wound blood flow in the animal model was also unaffected by changes in oxygen delivery (Table 4), suggesting that, while tissue hypoxia may be a potent stimulus for revascularization of the wound, it would not explain any vasodilatation of these new vessels.

Work has just begun to determine the neurogenic control of wound blood flow in this model. The problem is to be approached by calculating vascular resistance (perfusion pressure/blood flow) in injured and noninjured limbs during a variety of situations which alter peripheral vascular tone; i.e., hemorrhage, systemic release of circulating vasoactive substances and spinal blockade. The basic question

4. Wilmore DW, Aulick LH, Mason AD Jr, Pruitt BA Jr: Influence of the burn wound on local and systemic responses to injury. *Ann Surg* 186(4):444, 1977.

8. Äggård E, Jonsson C-E: Efflux of prostaglandins in lymph from scalded tissue. *Acta Physiol Scand* 81:440, 1971.

9. Edery H, Lewis GP: Kinin forming activity and histamine in lymph after tissue injury. *J Physiol (London)* 169:568, 1963.

10. Arterson G: Prostaglandins in human burn-wound secretion. *Burn* 3:112, 1977.

TABLE 4

	<u>Room Air</u>	<u>100% O₂</u>
Rectal Temperature (°C)	39.4 ± 0.1	39.2 ± 0.1
Arterial Po ₂ (mmHg)	92.4 ± 13.4	455 ± 23***
Uninjured Leg Blood Flow (ml/min)	64 ± 15	65 ± 9
Injured Leg Blood Flow (ml/min)	116 ± 14	123 ± 10

*** p < 0.001

to be asked is: does the increased blood flow to the injured limb reflect any decrease in neurogenic vasoconstrictor tone or other alteration of vascular smooth muscle sensitivity to known circulating vasoconstrictors?

SUMMARY AND CONCLUSIONS

The granulating wound, which develops 9-12 days post-surgical removal of the skin from one hindlimb of a goat, has many functional similarities to the surface wounds of burned patients. Blood flow to the injured limb (goat and patient) is markedly increased while that to the contralateral, uninjured limb remains normal. This increase in peripheral blood flow, presumably directed to the surface wound, is not in response to increased aerobic metabolism, but associated with an increase in glucose consumption and lactate production. Since all of these peripheral circulatory and metabolic adjustments to surface injury are identical to those observed in the thermally injured patient, the present goat model appears to be an appropriate means for the study of the control of burn wound blood flow.

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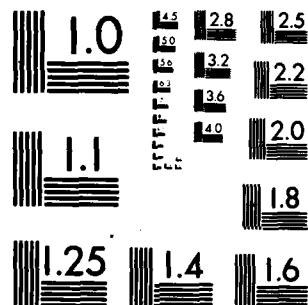
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Unclassified

ABSTRACT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS -- STUDIES OF HEPATIC BLOOD FLOW AND SUBSTRATE TURNOVER FOLLOWING THERMAL INJURY

US Army Institute of Surgical Research, Brooke Army Medical Center,
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Period covered in this report: 1 October 1977 - 30 September 1978

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Reports Control Symbol MEDDH-288(R1)

The liver is central to many metabolic responses and alterations in substrate flow which occur following injury. Hepatic blood flow studies by Gump and associates in three noninfected burn patients suggest that liver blood flow is increased following thermal injury, but that splanchnic blood flow is not elevated in proportion to the cardiac output of the patients (1). Measurements of extremity blood flow by Aulick, et al, indicate that approximately two-thirds of the extra cardiac output is directed to the burn wound (2). Studies of glucose kinetics in burn patients demonstrate that accelerated hepatic glucose production occurs with the wound selectively consuming glucose to facilitate tissue repair (3,4). These investigations further suggest that burn patients with gram negative bacteremia have impaired liver function which reduces the high rate of hepatic

1. Gump FE, Price JB, Kinney JM: Blood flow and oxygen consumption in patients with severe burns. Surg Gynec Obstet 130:23-28, 1970.

2. Aulick LH, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Influence of the burn wound on peripheral circulation in thermally injured patients. In press, Am J Physiol.

3. Wilmore DW, Mason AD Jr, Pruitt BA Jr: Alterations in glucose kinetics following thermal injury. Surg Forum 26:81-83, 1975.

4. Wilmore DW, Aulick LH, Mason AD Jr, Pruitt BA Jr: The influences of the burn wound on local and systemic response to injury. Ann Surg 186:444-458, 1977.

gluconeogenesis and alters the normal relationship between hepatic substrate production and wound consumption of these fuels (5).

In this study splanchnic blood flow and arterial-hepatic vein substrate concentration differences are measured; regional circulation and oxygen consumptions are compared with cardiac output and total body aerobic demands.

METHODS

Six noninfected burn patients have been studied to date. The average age was 28 years, weight 82.1 kg and body surface area 1.96 m². The mean total body surface burn of the six patients was 42%. The patients were studied in the stable hypermetabolic phase, one to two weeks postinjury, following uncomplicated resuscitation; the average time of study was on the eleventh postburn day.

After six hours of fasting the patient undergoing study was taken to the special procedure room in the Radiology Department and a 7F Cournand catheter placed in the hepatic vein via the femoral vein, using fluoroscopic visualization. The patient was then moved into the metabolic room (ambient temperature 30°C, R.H. 50%) and the subject positioned comfortably supine in bed. An arterial line was placed in an available peripheral artery, and an intravenous cannula inserted, if one was not already in place as required for clinical care. After at least 30-60 minutes of undisturbed rest, arterial and hepatic venous blood samples were drawn. Indocyanine green dye (0.5 mg/kg) was then given intravenously by bolus injection and serial timed arterial and hepatic vein samples drawn over the next 12-15 minutes for the calculation of hepatic blood flow (6). Cardiac output and oxygen consumption were measured by standard techniques previously described (4).

RESULTS AND DISCUSSION

In this group of six febrile hypermetabolic patients with a hyperdynamic circulation, hepatic (splanchnic) blood flow, oxygen consumption and net glucose production were elevated when compared with reported normal values (Table). The increase in splanchnic net perfusion was not proportional to the increase in cardiac output and hepatic blood flow represented a smaller fraction of the total cardiac output (16.7%)

5. Wilmore DW, Mason AD Jr, Pruitt BA Jr: Impaired glucose flow in burn patients with gram-negative sepsis. Surg Gynec Obstet 143: 720-724, 1976.

6. McDougal WS, Wilmore DW, Pruitt BA Jr: Glucose dependent hepatic membrane transport in nonbacteremic and bacteremic thermally injured patients. J Surg Res 22:697-708, 1977.

4. Wilmore DW, Aulick LH, Mason AD Jr, Pruitt BA Jr: The influences of the burn wound on local and systemic response to injury. Ann Surg 186:444-458, 1977.

Table - Systemic and Regional Circulatory and Metabolic Responses in Six Nonseptic Burn Patients (Mean)

	<u>Patients</u>	<u>Normal Values</u>
<u>Systemic Responses</u>		
Rectal Temperature ($^{\circ}\text{C}$)	38.3	37.0
Pulse Rate (beats/min)	107	75-85
Blood Pressure (mmHg)	128/72	120/80
Oxygen Consumption (ml/min) (ml/min \cdot m 2)	412 209	230-250 115-125
Metabolic Rate (kcal/hr \cdot m 2)	59.6	35-40
Cardiac Output (L/min)	15.09	5.0-5.5
Cardiac Index (L/min \cdot m 2)	7.76	3.0-3.2
<u>Regional Responses</u>		
Estimated Hepatic Blood Flow(EHBF) (L/min) (L/min \cdot m 2)	2.502 1.279	1.25-1.40 0.63-0.85
EHBF as % of Total Cardiac Output	16.7%	25%
Arterial-Hepatic Vein O $_2$ Difference (ml/100 ml)	4.6	4.5
Hepatic Oxygen Consumption (ml/min)	113	60-65
Hepatic V O_2 as % of Total V O_2	27.7	24-27
Arterial-Hepatic Vein Glucose Difference (mg/100 ml)	-8.2	-8 to -10
Hepatic Glucose Production (mg/min \cdot kg) (gm/day)	2.56 283	1.5-1.8 180
	378	

than normal (25%). This is similar to the earlier finding of Gump and co-workers in three burn patients (1). In addition, splanchnic blood flow appeared to increase in proportion to local aerobic demands; oxygen extraction was a 4.6 ml/100 ml, similar to the mean arterial-hepatic vein oxygen difference of 4.5 reported by Myers in a series of 63 hepatic vein catheterizations in normals (7).

The increased rate of hepatic glucose production is consistent with the results of earlier studies (3,4) which not only demonstrated an increase in glucose kinetics following injury but indicated that a major portion of this glucose is consumed by the burn wound. Subsequent studies will attempt to partition hepatic glucose output into that provided by glycogenolysis and gluconeogenesis.

These investigations are very preliminary and will be continued and expanded to include septic burn patients.

1. Gump FE, Price JB, Kinney JM: Blood flow and oxygen consumption in patients with severe burns. Surg Gynec Obstet 130: 23-28, 1970.

7. Meyers JD: The circulation of the splanchnic area. Transactions of the 4th Conference on Shock and Circulatory Hemostasis, New York, Josiah May Jr Foundation, 1955.

Splanchnic blood flow

Visceral perfusion and metabolism

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF
BURN INJURY IN SOLDIERS -- THE RELATIVE SIGNIFICANCE
OF THERMAL AND METABOLIC DEMANDS ON BURN HYPERMETABOLISM

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To evaluate the claim that burn hypermetabolism can be eliminated by making patients warm and comfortable, respiratory gas exchange was measured, using a canopy hood system, in 20 burn patients and 4 normal controls resting in a 30°C ambient environment. The metabolic rates of burn patients, determined by the canopy hood system, were comparable to previous values obtained using Douglas bag techniques. In another study, the effects of induced hyperthermia were evaluated in 7 febrile, hypermetabolic burn patients. External heating elevated rectal temperatures from 38.6 ± 0.3 to 39.5 ± 0.2 (mean \pm S.E.M., $P < 0.001$) but did not significantly reduce metabolic rate (76.5 ± 3.7 kcal/m² · hr vs 73.9 ± 3.5). Finally, 5 normals and 7 burn patients adjusted room temperature until they were comfortable. The "preferred" environmental temperature selected by the burn patients was $31.5 \pm 0.7^\circ\text{C}$, significantly warmer than the $28.6 \pm 0.7^\circ\text{C}$ selected by the controls. In this comfortable environment the sleeping, febrile burn patients remained hypermetabolic (63.9 ± 5.7) and the Q_{10} effect of the hyperpyrexia accounted for only 20-30% of the hypermetabolism observed.

These studies demonstrate that the hypermetabolic response to burn injury is not significantly reduced in comfortable patients resting or sleeping in a warm environment, and the increased oxygen consumption cannot be accounted for by the elevated body temperature. This evidence does not support the contention that burn hypermetabolism is primarily the result of thermoregulatory drives, but reconfirms the thesis that the increased heat production following injury is the consequence of an elevated metabolic state.

Oxygen consumption
Ambient temperature

THE RELATIVE SIGNIFICANCE OF THERMAL AND METABOLIC
DEMANDS ON BURN HYPERMETABOLISM

Hypermetabolism characterizes the metabolic response to thermal injury. A variety of investigations have demonstrated that the increased oxygen consumption is not the result of post traumatic hyperthyroidism (6,8) but rather is mediated by increased activity of the sympathetic nervous system (15,22). Originally the elevated heat production was considered to be a thermoregulatory response to the increased evaporative heat loss from the surface wound (14,18). Moyer was the first to emphasize this relationship and reported that the metabolic rate of one patient was reduced following immersion in a warm saline bath (17). Other evidence from animal studies supported this thesis: metabolic rate in a burned rat was reduced when evaporative water loss was blocked (16) and rats with a 20% burn were hypermetabolic at a 20°C room temperature but not when placed in a 30°C environment (17). A frequently cited clinical report in support of the evaporative cooling thesis is that of Barr et al (5). Metabolic rates reported in two patients studied in a 22°C room ranged between 60-160% above basal. Treated in a 32°C room two other

6. Becker R, Johnson DW, Woeber KA, et al: Depressed serum triiodothyronine (T₃) levels following thermal injury. *Fed Proc* 35: 216, 1976.

8. Cope O, Nardi GL, Quijano M, et al: Metabolic rate and thyroid function following acute thermal trauma in man. *Ann Surg* 137: 165-174, 1953.

15. Harrison TS, Seaton JS, Feller J: Relationship of increased oxygen consumption to catecholamine excretion in thermal burns. *Ann Surg* 165:169-172, 1964.

22. Wilmore DW, Long JA, Skreen R, et al: Catecholamines: Mediator of the hypermetabolic response to thermal injury. *Ann Surg* 180:653-668, 1974.

14. Harrison HN, Moncrief JA, Duckett JW, et al: The relationship between energy metabolism and water loss from vaporization in severely burned patients. *Surg* 56:203-211, 1964.

18. Roe CF, Kinney JM: Water and heat exchange in third-degree burns. *Surgery* 56:212-220, 1964.

17. Moyer CA: The metabolism of burned mammals and its relationship to vaporizational heat loss and other parameters. In Research in Burns, Washington, DC. Am Inst Biol Scien, Philadelphia, 1962, F A Davis Co #9, p 113.

16. Lieberman ZH, Lansche JM: Effects of thermal injury on metabolic rate and insensible water loss in rats. *S Forum* 7:83-88, 1957.

5. Barr P-O, Birke G, Liljedahl S-O, et al: Oxygen consumption and water loss during treatment of burns with warm dry air. *Lancet* 1:164-168, 1968.

patients "demonstrated a substantial reduction in metabolic rate" and "peak values for BMR did not exceed 75% above normal for the extensive burn [a patient with a 60% total body surface injury] and 50% for the smaller burn [a 30% total body surface burn]..."

Although these studies strongly suggest a thermoregulatory basis for postburn hypermetabolism, additional work from our Institute questions the significance of environmental factors as the sole stimulus. Evaporative cooling of the wound does not appear to be a major metabolic stimulus in a thermal neutral environment, since Zawacki et al (25) were unable to demonstrate any consistent change in the metabolic rates of burn patients in such an environment after blocking evaporative water loss by wrapping the wound in a water impermeable membrane. In another study, oxygen consumption was determined in burn patients in a variety of ambient temperatures (19-33°C) (22). Metabolic rate decreased as ambient temperature increased and the extent of this environmental effect was burn size related. However metabolic rate remained markedly elevated even when ambient temperature was increased up to and above thermal neutrality, suggesting that thermoregulatory factors in this warm environment were not the dominant stimulant of burn hypermetabolism, but the increased heat production was the consequence of an elevated metabolic state.

The interaction between evaporative water loss, ambient temperature and hypermetabolism has yet another facet which must be considered. Burn patients are febrile and appear to thermoregulate around an elevated central reference temperature (4). They maintain above normal surface and central body temperatures over a wide range of thermal environments (19-33°C) (22,23). Because of a rise in "set point" temperature, these febrile burn patients prefer above normal ambient temperatures (30-33°C) to achieve thermal comfort (24). In

25. Zawacki BE, Spitzer KW, Mason AD Jr, et al: Does increased evaporative water loss cause hypermetabolism in burned patients? *Ann Surg* 171:236-240, 1970.

22. Wilmore DW, Long JA, Skreen R, et al: Catecholamines: Mediator of the hypermetabolic response to thermal injury. *Ann Surg* 180:653-668, 1974.

4. Aulick LH, Wilmore DW: Thermal regulation in burn patients. In Allison S (Ed): Metabolic Response to Stress, London, Academic Press. In press.

23. Wilmore DW, Mason AD Jr, Johnson DW, et al: Effect of ambient temperature on heat production and heat loss in burn patients. *J Appl Physiol* 38:593-597, 1975.

24. Wilmore DW, Orcutt TW, Mason AD Jr, et al: Alterations in hypothalamic function following thermal injury. *J Trauma* 15:697-703, 1975.

this warm environment, unburned skin remains relatively vasoconstricted as an additional means to satisfy the new hypothalamic reference temperature and maintain the febrile state (2,3). However, under these conditions of comfort, burn patients continue to maintain metabolic rates $1\frac{1}{2}$ -2 times basal. Thus, while there may be thermoregulatory influences on burn hypermetabolism, the increased rate of heat production is primarily determined by metabolic factors; that is, burn hypermetabolism is temperature sensitive but not temperature dependent.

This concept is not universally accepted, however. Recently, Swedish investigators have stated that postburn hypermetabolism can be eliminated by allowing the patient to control the amount of radiant heat received from infrared lamps located over the bed (11). Through the use of a bedside control unit, their patients selected a comfortable thermal environment and then usually fell asleep. Metabolic rates were determined by indirect calorimetry using a flow through canopy hood system (10). Under these conditions, the metabolic rates of sleeping patients were considerably lower than those reported from this Institute in a similar group of patients resting quietly in a 33°C room (22). These Swedish investigators attributed this difference to the more standard approach of respiratory gas collection (Douglas bags) utilized in our earlier study. They suggested that such an approach provided for a systematic increase in the metabolic rate because our patients were not asleep and, most likely, uncomfortable when using the mouthpiece and nose-clip.

Contrary to stated claims, their data reveal that sleeping burn patients remained hypermetabolic: the degree of hypermetabolism was said to vary as a function of core temperature but not burn size. When oxygen consumption was corrected by reducing metabolic rate by

2. Aulick LH, Wilmore DW, Mason AD Jr, et al: Influence of the burn wound on peripheral circulation in thermally injured patients. *Amer J Physiol* 237:901-906, 1977.

3. Aulick LH, Wilmore DW, Mason AD Jr, et al: Elevated central reference temperature following thermal injury, *Proceedings of the International Union of Physiological Sciences* 13:37, 1977.

11. Danielsson U, Arturson G, Wennberg L: The elimination of hypermetabolism in burned patients. *Burns* 2:110-114, 1976.

10. Danielsson U, Arturson G, Wennberg L: A new technique for the long-term stable measurement of energy expenditure. *Burns* 2: 107-109, 1976.

22. Wilmore DW, Long JA, Skreen R, et al: Catecholamines: Mediator of the hypermetabolic response to thermal injury. *Ann Surg* 180:653-668, 1974.

16% per °C rise in rectal temperature above 37, the adjusted rate of metabolism was near normal. While the significance of this mathematical manipulation is difficult to appreciate, the implication of the work is that burn hypermetabolism can be eliminated by making the patient warm and comfortable. As this is in direct contrast with all reported clinical observations and interpretations of burn hypermetabolism, a series of experiments were designed to re-examine this problem. This paper describes the use of a similar noninvasive measurement system for determination of respiratory gas exchange in sleeping patients, validates this technique and utilizes this system to determine energy expenditure in burn patients under a variety of environmental conditions.

MATERIALS AND METHODS

1) The Measurement System

This system consists of four separate components; the head box for expiratory gas collection, a gas analyzer, a flowmeter, and a computational system. The rigid head box, made of clear plexiglas, is modeled after a previously described canopy hood design (19). The subject places his head through a large opening in the front of the head box and a pliable loose-fitting neck seal is tucked around the neck and shoulders and held in place by a velcro band or folded towel. The top of the box, removable to allow access to the patient's face, is held in place with tension springs. Air enters the hood around the neck seal and through a small port in the upper corner of the box. Room air is pulled through the box by an exhaust fan, insuring that all expired air exits through the exhaust opening located behind the patient's head (Fig 1). Just distal to the exhaust fan is an aperture which may be opened or closed manually, regulating the rate of air flow through the box. Due to individual variations in metabolism, flow rate is adjusted for each subject, and ranged from 40 liters/min for control subjects to 70 liters/min for some hypermetabolic patients. Optimizing air flow through the box minimizes the accumulation of carbon dioxide within the canopy hood and maximizes the difference between inlet and exit oxygen concentrations, thus increasing the accuracy of the gas determinations.

Gas flow through the head box is measured directly behind the exhaust fan by a low resistance turbine flowmeter (Quantum Dynamics Model QL-12R). This flowmeter provides a linear voltage response to flow from 0-500 liters/minute.

19. Spencer JL, Zikria BA, Kinney JM, et al: A system for continuous measurement of gas exchange and respiratory functions. J Appl Physiol 33:523-528, 1972.

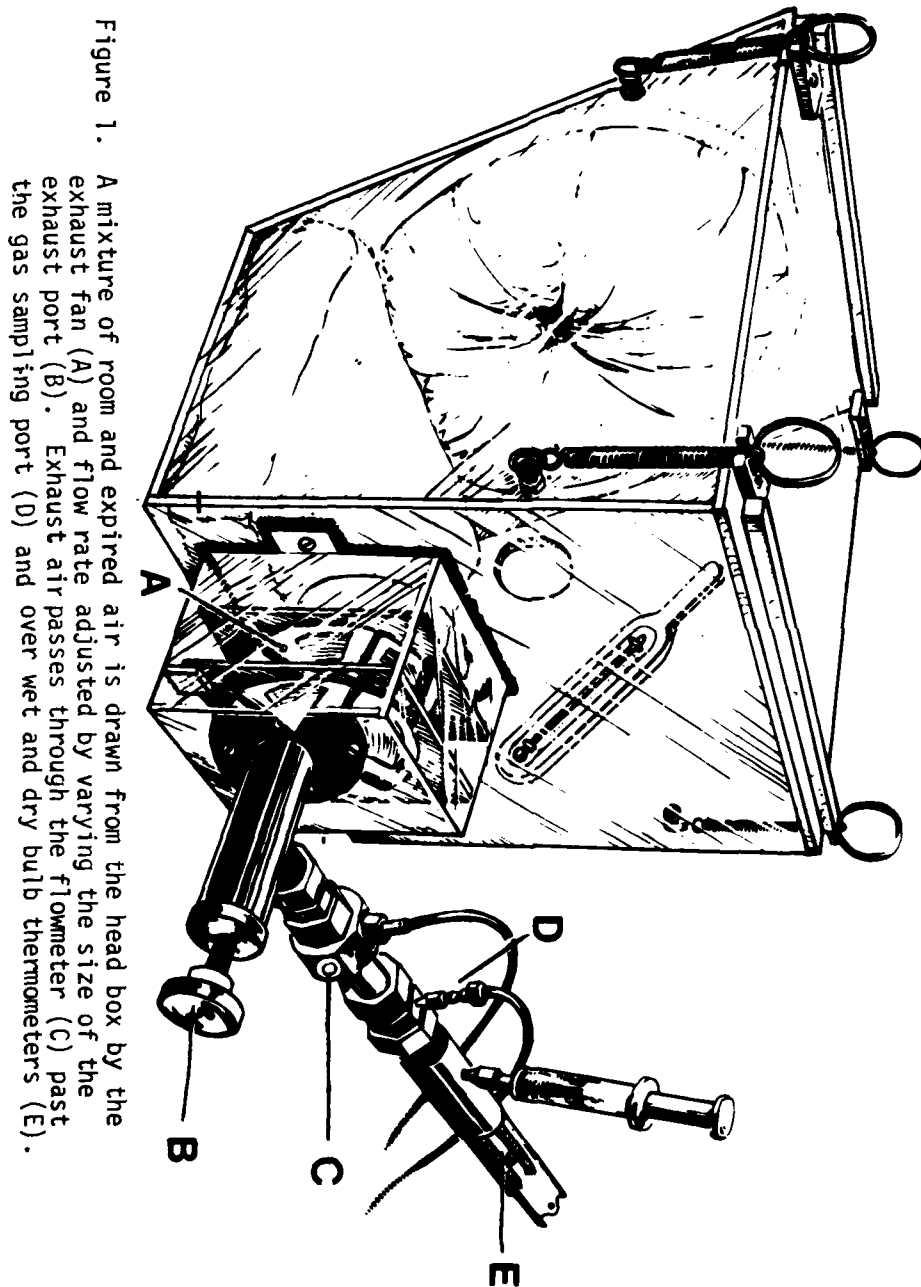


Figure 1. A mixture of room and expired air is drawn from the head box by the exhaust fan (A) and flow rate adjusted by varying the size of the exhaust port (B). Exhaust air passes through the flowmeter (C) past the gas sampling port (D) and over wet and dry bulb thermometers (E).

Gas analysis is provided by a Perkin-Elmer Model MGA 1100 Medical Gas Analyzer. This device is a fixed magnetic sector mass spectrometer which provided continuous analysis of oxygen, carbon dioxide, nitrogen and water vapor in the exhaust gas stream, producing high level analog signals with a low drift rate.

The computation system consists of a Digital Equipment Corporation LSI-11 microcomputer system with 8K words of random access memory, line frequency clock, and interfaces to both the terminal and the analog conversion system. Analog signals from the mass spectrometer and flowmeter are entered into the microcomputer by a 12 bit analog-to-digital converter, interfaced through a 16 bit parallel I/O board.

The terminal (Texas Instruments Silent 700), interfaced with the microcomputer through a 300 baud serial line, provides a serial record of the subject's oxygen consumption, carbon dioxide production, respiratory exchange ratio, metabolic rate, average expired O_2 and CO_2 fractions, and average exhaust air flow rate. Normal sample interval ranged between two and five minutes.

The program, written in assembly language, resides in core memory and thus is semipermanent. The program requires about 3600 words of permanent storage and 1000 words of scratch pad storage. If program correction or reloading is required, this is accomplished from a DEC PDP-11/40 computer across a dial-up telephone connection.

2) Computational Procedure

Utilizing equations previously described (10,19), oxygen consumption and carbon dioxide production are computed by multiplying the difference between inlet and exhaust gas concentrations times the rate of gas flow. The gas volumes are corrected to standard temperature, pressure and dry conditions. Temperature and water vapor pressure of the exhaust gas and barometric pressure are measured at the start of each run and entered into the program to permit this conversion.

Validation of this system was performed by determining the metabolic rate of normals under basal and exercise conditions using this system and simultaneously collecting the exhaust gas from the head box in a set of Douglas bags. Gas concentrations

10. Danielsson U, Arturson G, Wennberg L: A new technique for the long-term stable measurement of energy expenditure. Burns 2: 107-109, 1976.

19. Spencer JL, Zikria BA, Kinney JM, et al: A system for continuous measurement of gas exchange and respiratory functions. J Appl Physiol 33:523-528, 1972.

and volume were then measured in the bags and the results compared with those values provided by the flow-through measurement system.

3) Patient Studies

In the first phase of the clinical study, 20 noninfected burn patients were selected to represent a wide range of total body surface injuries (from 9.5-86% total body surface injury). Mean burn size for this group of patients was 44% total body surface; all were male, and none had known pre-existing diseases. The patients were studied between the seventh and twenty-second post-burn day, with the mean day of study being the thirteenth day following injury. All patients were: a) normotensive and hemodynamically stable, b) in a normal state of hydration, with a hematocrit greater than 33, and without abnormalities in serum electrolyte concentration, osmolality, or pH, c) free of systemic infection as determined by clinical symptoms and signs, chest x-rays, and daily blood cultures, and d) alert, cooperative, and able to participate in the study.

Five experienced subjects, males of comparable age and body size, served as controls. All subjects were studied in the early morning. Normal individuals were fasted for at least 10 hours before the study, and all patients were fasted after midnight. Those patients requiring intravenous fluid to maintain a normal state of hydration received 0.04 molar, nutrient-free sodium chloride infusions for six hours before and throughout the study. While routine clinical care continued, patient manipulation was minimized for at least six hours before and during the study.

All studies took place in an environmental chamber previously described (23). The ambient temperature was maintained at 30°C, and relative humidity ranged between 40-50%. Control subjects wore light cotton shorts, and patients were draped with a light cotton towel. The subjects were moved to the study room between 5:00 and 6:00 A.M. and placed supine in bed. A rectal thermocouple probe was inserted and core temperature monitored at five-minute intervals throughout the study. After an initial equilibration period, the canopy hood was placed over the patient's head and continuous oxygen consumption and carbon dioxide production measured over 15-30 minutes. These data were compared with a set of respiratory gas

23. Wilmore DW, Mason AD Jr, Johnson DW, et al: Effect of ambient temperature on heat production and heat loss in burn patients. J Appl Physiol 38:593-597, 1975.

measurements previously reported, using the standard Douglas bag techniques in patients prepared for study in the same manner (22).

In the second phase of the clinical evaluation, seven patients were studied to determine the impact of environmental heating on the post traumatic hypermetabolic response. The mean burn size for this group averaged 65% total body surface and ranged from 40.5-86%. The patients were studied during the second week of injury; the mean day of study was the 11th postburn day, and ranged from 8-15 postburn days. None of the patients were septic or had previously documented bacteremia. The patients were prepared as previously described and, on the morning of the study, were moved into the environmental chamber, maintained at 30°C, relative humidity 40-50%. After the patients were placed supine in bed, a rectal probe was inserted to a depth of 10 cm from the rectal sphincter and nine to 13 thermocouples were attached to the skin at the same sites in all subjects. These core and surface temperatures were recorded at five minute intervals in order to identify the establishment of thermal equilibrium prior to external heating. Rectal temperature was monitored during the heating phase to insure that levels of induced hyperthermia were not excessive.

After at least two hours of essentially undisturbed rest in the environmental room, the patient's head was slipped into the head box and a series of consecutive metabolic determinations initiated. Each determination was based on the integration of exhaust gas concentrations and flow rate for the preceding two minutes. This series of continuous, on line data analyses continued until a steady-state metabolic rate was apparent and then the patient was heated. In two studies, this was accomplished by raising the ambient temperature of the metabolic room to 35-37°C. In the remaining five patients, three radiant heat lamps were placed over the patients and served as the external heat source. The elevated environmental temperature was maintained from 30 minutes to three hours and was discontinued when the patient complained of being too warm, had a rectal temperature above 40°C, or reached a new steady state body temperature and metabolic rate. While all subjects rested quietly throughout the study, five of the seven patients slept.

In the third phase of the clinical study, seven patients and five controls were prepared as previously described and moved into the environmental chamber set at 30°C, relative humidity 40-50%.

22. Wilmore DW, Long JA, Skreen R, et al: Catecholamines: Mediator of the hypermetabolic response to thermal injury. *Ann Surg* 180:653-668, 1974.

The subjects were instructed to use a bedside temperature controller and set ambient temperature to their comfort level. After two to six hours of adjusting the ambient temperature, the subjects found their preferred thermal environment and usually went to sleep. At that time, the canopy hood was placed over the subject's head and metabolic rate monitored until stable. At that point, the subject was awakened, rectal temperature measured with a standard mercury thermometer and the study terminated.

RESULTS

The canopy hood system for measuring gas exchange yielded results comparable to those determined by simultaneous Douglas Bag collections (Fig 2), confirming the validity of this measurement technique.

In the 30°C ambient environment, the metabolic rates of sleeping burn patients increased in a curvilinear fashion with burn size and reached a plateau as the extent of total body surface injury exceeded 40-50% (Table 1, Fig 3). The equation which describes this relationship between resting metabolism and burn size was comparable to a general predictor formula established from the previously reported Douglas bag data (22). Therefore, essentially no difference in metabolic expenditure could be ascertained in burn patients between studies using the canopy hood system and those utilizing the Douglas bag technique.

In the second phase of this study, heating febrile, hypermetabolic burn patients increased rectal temperature on the average of 0.8°C. Individual responses to induced hyperthermia were quite variable (Table 2, Fig 4). Three subjects decreased heat production approximately 14%; one increased heat production approximately 23%, and the other three subjects demonstrated no alteration in metabolic activity. In this group, therefore, average resting metabolic rate was not altered by environmental heating. At no time was the metabolic rate returned to normal by the elevation in body temperature.

Finally, when the burn patients were allowed to select their "preferred" ambient temperature, they consistently chose a room temperature which was above that selected by the normal individuals (Table 3). In this environment, the subjects slept and the metabolic

22. Wilmore DW, Long JA, Skreen R, et al: Catecholamines: Mediator of the hypermetabolic response to thermal injury. *Ann Surg* 180:653-668, 1974.

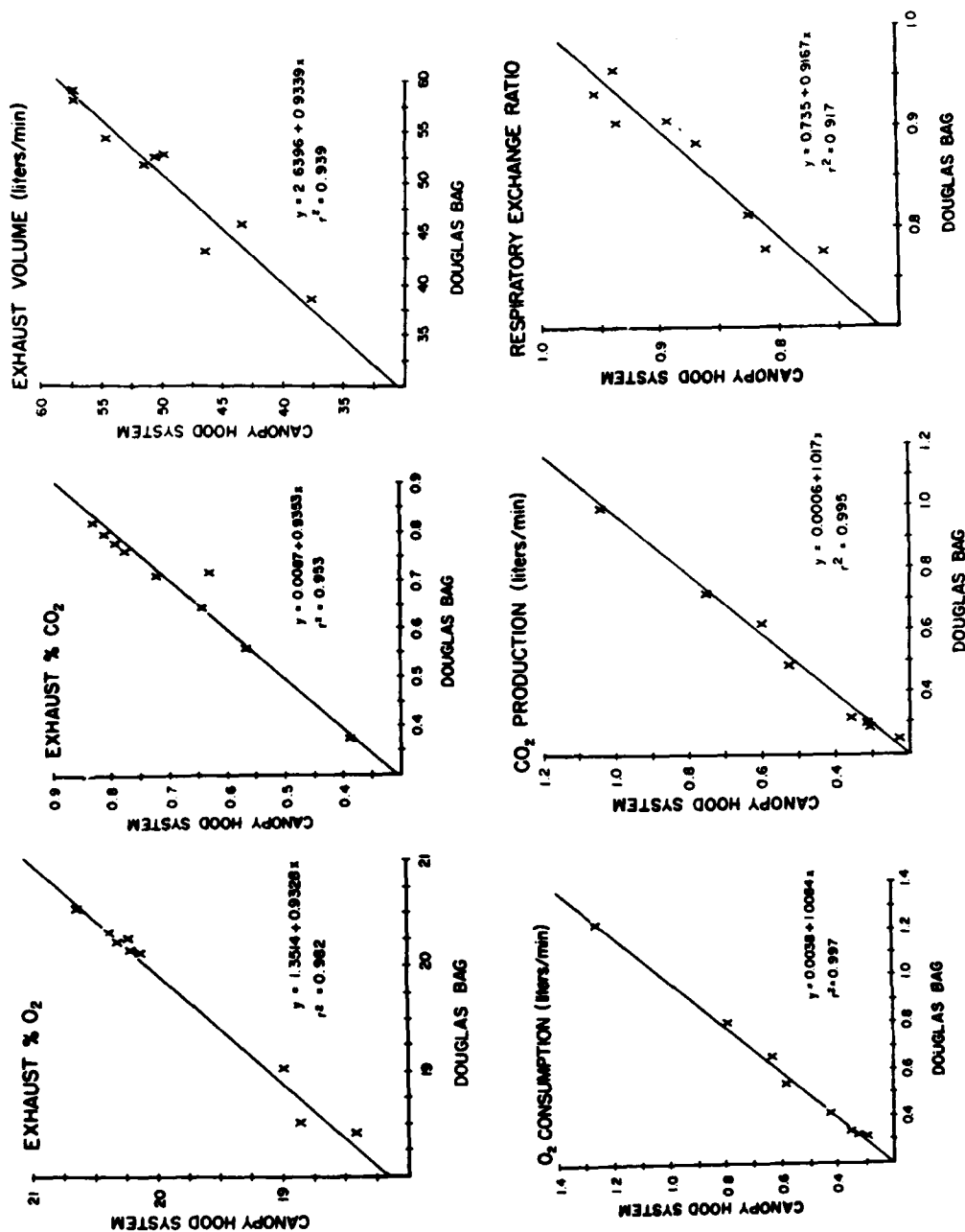


Figure 2. Comparison of Douglas bag and canopy hood techniques for the measurement of respiratory gas exchange of resting and exercising normals. Levels of bicycle exercise were selected to produce metabolic rates comparable to those observed in resting burn patients.

TABLE 1
Characteristics, Metabolic Rate and Rectal Temperature
of Resting Subjects Studied in a 30°C Environment

Subject	Age (Years)	Weight Kg	Body Surface Area (m ²)	% Total Body Surface Burn	Postburn Day Studied	Metabolic Rate (Kcal/m ² ·hr)	Rectal Temperature (°C)
Controls							
1	24	75.0	1.98	0	-	37.0	37.0
2	35	77.3	2.06	0	-	36.5	37.1
3	29	84.1	2.04	0	-	37.0	36.8
4	28	63.6	1.72	0	-	33.9	36.9
5	38	77.3	2.06	0	-	36.6	36.6
Patients							
1	22	75.0	1.76	9.5	8	50.0	37.4
2	19	62.0	1.74	10.5	10	47.9	37.5
3	50	58.6	1.72	12	10	41.2	36.7
4	36	93.1	2.18	17.5	12	50.7	37.6
5	18	79.4	1.88	25.5	8	59.0	38.6
6	32	89.4	2.05	29	9	59.2	38.0
7	54	76.3	1.92	29.5	12	54.4	39.4
8	18	69.6	1.91	35	11	55.3	37.8
9	45	80.6	2.00	40.5	8	81.6	39.0
10	40	88.0	2.10	45	12	55.6	38.1
11a	22	67.2	1.72	46.5	8	64.5	39.4
b	22	57.5	1.63	46.5	19	51.8	39.3
12	18	80.3	2.07	50	22	59.7	38.3
13	20	59.1	1.70	50.5	10	78.9	38.7
14	19	67.1	1.86	52	10	62.5	38.7
15	17	82.5	2.05	57.5	14	74.1	39.0
16	19	68.2	1.91	61.5	21	87.6	39.6
17	19	64.4	1.73	67	11	59.1	37.2
18	21	67.2	1.78	73	15	70.4	38.7
19	24	53.4	1.61	78	20	64.4	38.5
20	22	79.5	2.10	86	8	76.9	37.5

TABLE 2 THE EFFECT OF INDUCED HYPERTHERMIA ON METABOLIC RATE OF BURN PATIENTS

Subject	Age (Years)	Weight (kg)	Body Surface Area (m ²)	% Total Body Surface Burn	Postburn Day Studied	Before Heating†		After Heating	
						Metabolic Rate (Kcal/m ² ·hr)	Rectal Temp (°C)	Metabolic Rate (Kcal/m ² ·hr)	Rectal Temp (°C)
1	22	79.5	2.10	86	8	76.9	37.5	65.3	38.6
2	17	82.5	2.05	57.5	14	74.1	39.0	63.6	39.7
3	21	67.2	1.78	73	15	70.4	38.7	70.0	39.1
4	45	80.6	2.00	40.5	8	81.6	39.0	72.4	39.6
5	18	60.9	1.69	63	11	85.8	39.3	87.7	40.1
6	19	64.4	1.73	67	11	59.1	37.2	72.8	39.1
7	19	68.2	1.91	61.5	13	87.6	39.6	85.3	40.0

mean

±SE

*30°C environment

65 11 76.5 38.6 73.9* 39.5**

± 3.7

± 0.3

± 3.5

± 0.2

*No difference from control period
**p < 0.001 significantly different
from control period

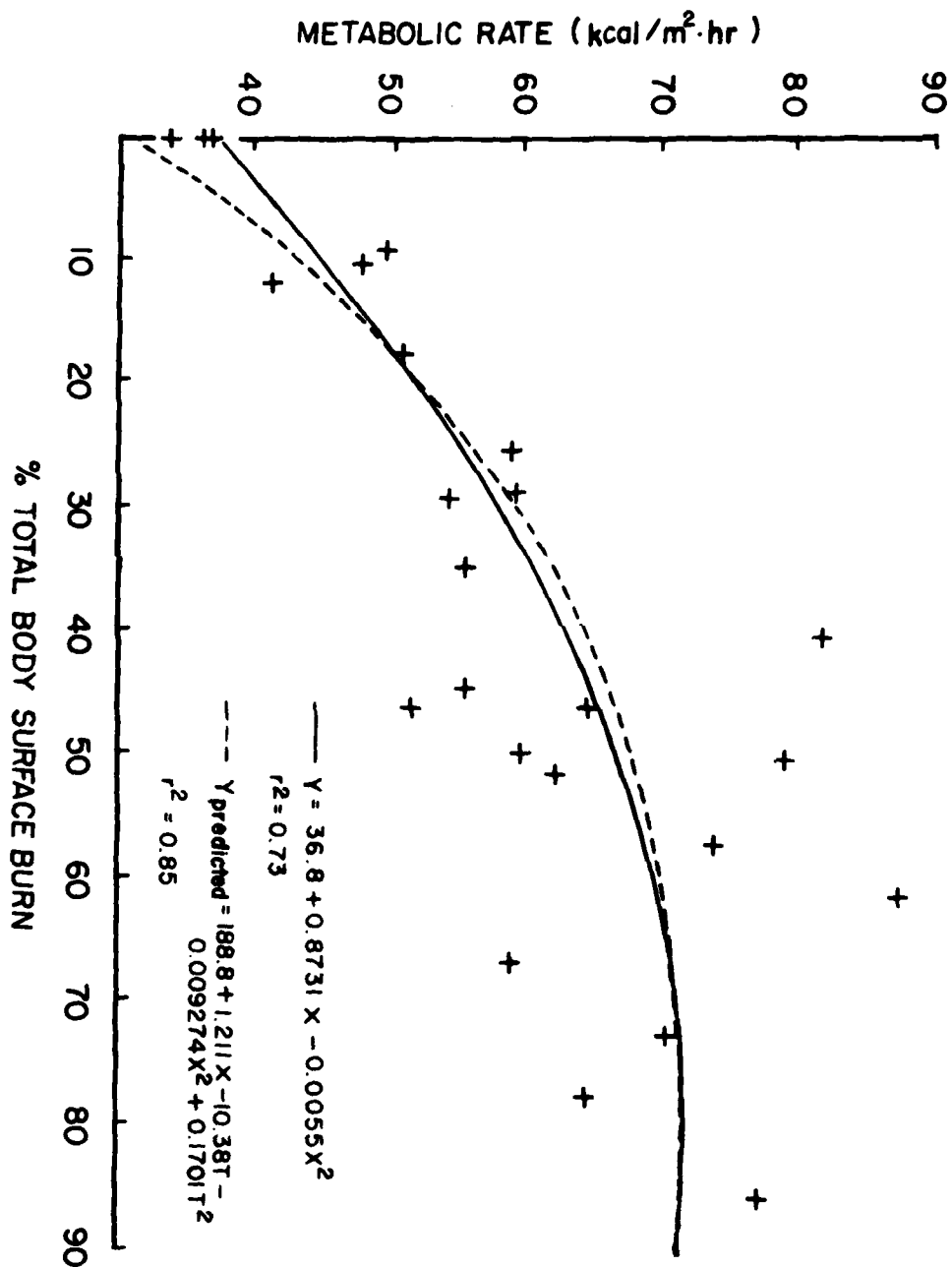


Figure 3. Metabolic rate of resting subjects, measured in a 30°C ambient environment by the canopy hood system, increases with burn size. This relationship is the same when metabolism is determined by the head box method (solid line is the best fit regression of the plotted data points) as that predicted from previous measurements (22) using the Douglas bag technique (broken line).

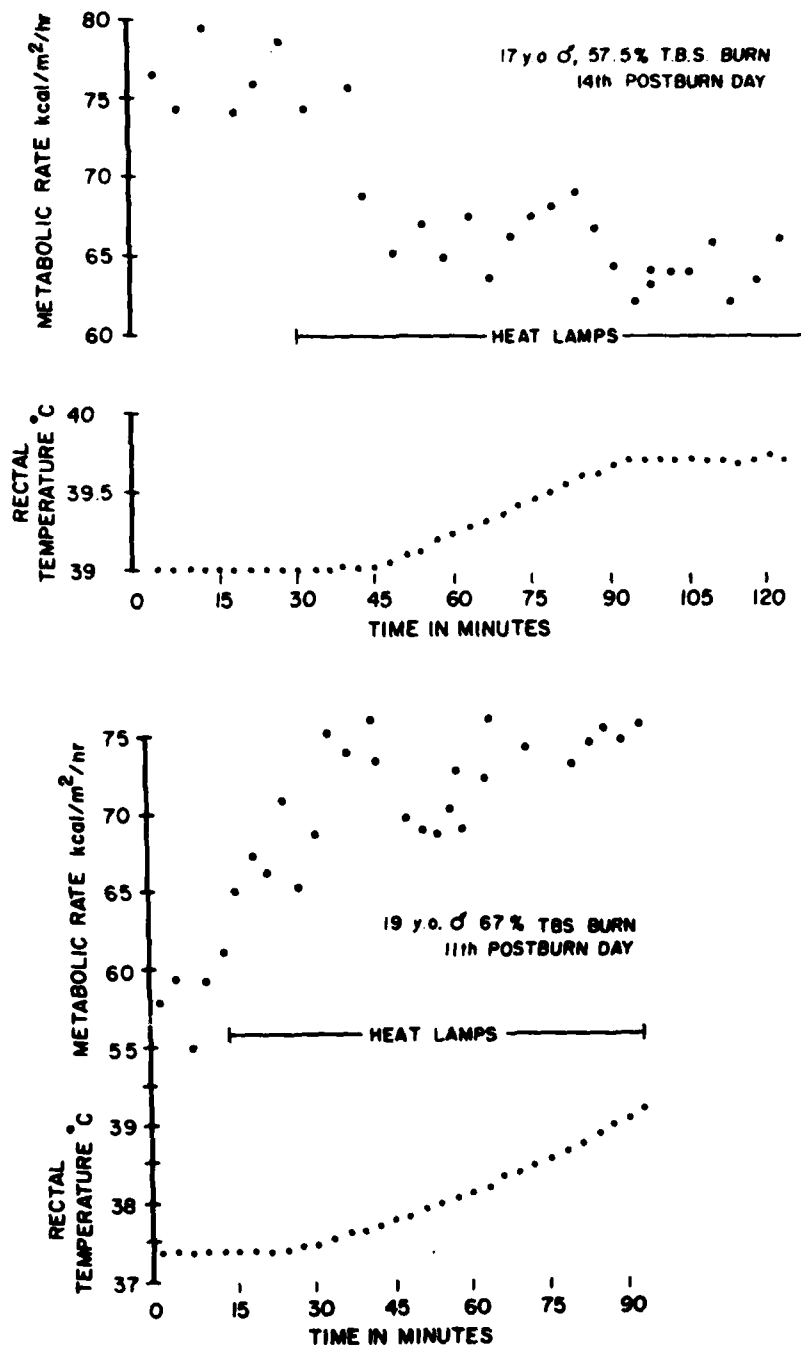


Figure 4. The extremes in the metabolic response to induced hyperthermia are demonstrated in these two patients. Continuous metabolic measurements (plotted every 2-3 minutes) reveal both the fluctuation in metabolic rate around a steady state in a 30°C environment and these dynamic responses to rising body temperatures. As illustrated in these two subjects, the metabolic response to radiant heating could not be predicted from initial rectal temperature.

Subject	Age (Yr)	Weight (Kg)	Body Surface Area (m ²)	% Total Body Surface Burn	Postburn Day Studied	Ambient Temperature Selected (°C)	Metabolic Rate (Kcal/m ² ·hr)	Rectal Temperature (°C)
Controls								
1	30	81.8	2.01	-	-	27.0	33.2	36.7
2	38	77.3	2.06	-	-	28.0	31.7	36.6
3	37	79.5	2.06	-	-	28.7	34.5	37.0
4	27	75.4	1.98	-	-	31.4	35.5	37.0
5	25	70.4	1.87	-	-	28.0	35.8	37.0
mean ± SE		31	76.9	2.00		28.6 ± 0.7	34.1 ± 0.8	36.9 ± 0.1
Patient								
1	42	83.2	2.08	19	7	28.5	47.3	37.2
2	52	67.7	1.79	22	11	30.5	44.5	37.0
3	29	80.2	1.95	32	18	32.5	62.0	38.6
4	30	89.1	2.15	37.5	10	33.1	63.2	37.0
5	25	65.7	1.66	44	10	31.0	84.2	38.9
6	24	96.2	2.00	49	7	33.6	65.5	39.2
7	21	70.4	1.93	56	14	31.4	80.7	38.9
mean ± SE		32	78.9	1.94	37	31.5 ± 0.7	63.9 ± 5.7	38.1 ± 0.4
						p < 0.05	p < 0.001	p < 0.01

rates of the burn patients were still significantly elevated above control values; this elevation in metabolism could not be accounted for by increased rectal temperature.

DISCUSSION

The results of this study fail to support the contention that burn patients are hypermetabolic because they are either cold or uncomfortable. The Swedish investigators contend that burn patients are extremely labile during manipulation and metabolic rate is consistently elevated during and following patient therapy (11). They postulate that spurious elevations in resting metabolic rate could occur while using a nose clip and mouthpiece for respiratory gas collection and suggest that a canopy hood measurement system is more desirable. Using this approach, burn patients can be studied while resting comfortably or even asleep. Such a measurement system was used in this study. The accuracy of the canopy hood system over the entire range of metabolic rates expected in normals and burn patients was first determined in resting and exercising normals. No major differences were observed when comparing the canopy hood system with standard Douglas bag technique (Fig 2). Confirmation of the accuracy of the canopy hood system used by the Swedish investigators was made in resting normals, but no reports of the accuracy or precision of their measurements were reported during periods of hypermetabolism.

The metabolic rates of burn patients determined by canopy hood system were compared with the predicted values obtained from previously reported Douglas bag measurements (22). No differences in the best fit curvilinear regressions of metabolic rate on burn size were observed when comparing the two techniques (Fig 3). Although the canopy hood system minimizes stimulation and allows patients to rest or sleep, the careful preparation and training of subjects and the study of selected individuals with tracheostomies in our earlier studies averted any systematic upward variation in metabolic rate resulting from anxiety or patient stimulation when using the nose clip and mouthpiece.

Are the post traumatic elevations in oxygen consumption primarily a response to increased body temperature as suggested by others? Van't Hoff described the physical chemical law that the rate of

11. Danielsson U, Arturson G, Wennberg L: The elimination of hypermetabolism in burned patients. *Burns* 2:110-114, 1976.

22. Wilmore DW, Long JA, Skreen R, et al: Catecholamines: Mediator of the hypermetabolic response to thermal injury. *Ann Surg* 180:653-668, 1974.

biochemical reactions increase with heating (20); for ordinary temperatures the velocity of chemical reactions increases approximately 2-3 fold for every 10° rise in temperature (Q_{10} effect). The significance of this Q_{10} effect has been considered by DuBois, who studied a group of patients with fever secondary to infectious disease (13). He noted that the elevation in rectal and body temperature resulted from elevations in metabolic rate; that is the hypermetabolism preceded the fever, the fever did not cause the hypermetabolism. The metabolic rate of a febrile, non-shivering subject without large protein losses could be predicted from rectal temperature; BMR increased approximately 13% per degree centigrade rise in rectal temperature above normal (consistent with a Q_{10} value between two and three).

The Swedish group contends that metabolic rate is best correlated with rectal temperature and not with burn size. Examining our data from the patients in the 30°C environment (all of whom were resting comfortably and most of whom were sleeping) metabolic rate was best correlated with percent total body surface burn ($r^2=0.73$). Although rectal temperature could be related to metabolic rate ($r^2=0.41$), this relationship occurred primarily because the normal controls were afebrile and the burn patients exhibited varying degrees of hyperthermia. To argue that the increase in body temperature following burn injury accounts for the elevation in metabolic rate is to suggest that hypermetabolism depends on or occurs because of the elevation in body temperature. While variations in body temperature undoubtedly affect metabolism, physiological studies in infected, febrile man clearly demonstrate that elevations in body temperature are the consequence, not the predominate cause of hypermetabolism (13). This cause and effect relationship also occurs in the burn patient. While internal heat production is essential to maintain the febrile state, our data demonstrate that metabolic drives, rather than these thermoregulatory demands on the patient, are the predominant causes of hypermetabolism. Increasing the ambient temperature satisfies the thermal drives but fails to abate burn hypermetabolism. If the mathematical correction suggested by others to account for the Q_{10} effect were applied to our own data, the hyperpyrexia would account for only 20-30% of the hypermetabolism observed. These calculations are similar to the reports of others (12).

20. Van't Hoff JH: Studies in chemical dynamics. Revised by Cohen E, Chemical Publishing Co, Easton, Pa, 1896.

13. Du Bois EF: The basal metabolism in fever. JAMA 77:352-355, 1921.

12. Davies JWL, Lamke L-O, Liljedahl S-O: Treatment of severe burns. Acta Chir Scand, Supp #468, p 56, 1977.

From a practical standpoint such an "on paper" correction of metabolic rate serves no real purpose in the clinical setting, for actual calorie requirements of the patients remain $1\frac{1}{2}$ -2 times basal levels. The recommendations of the Swedish investigators for calorie support (1) are quite similar to our suggested level of feeding (21). Weight loss is comparable in both groups of patients, suggesting that in spite of the metabolic measurements and their "correction" and interpretation, the Swedish burn patients and our burn patients actually require similar quantities of calories during the hyper-metabolic phase of injury even when patient controlled heaters are employed. Similar observations of calorie intake and weight loss in burn patients have been made by others (9) suggesting that from a treatment standpoint metabolic rate remains elevated at about twice normal levels. The patients should be treated in a warm environment of at least 30°C and, if possible, allowed to adjust the ambient temperature to preferred levels of comfort. Although this manipulation of the environment will minimize oxygen consumption, metabolic requirements remain markedly elevated and presumably reflect the energy costs required for wound healing and tissue repair.

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21. Wilmore DW: Nutrition and metabolism following thermal injury. In Moncrief JA (Ed): Clinics in Plastic Surgery, Philadelphia, W B Saunders, 1974, pp 603-619.

9. Curreri PW, Richmond D, Marvin J, et al: Dietary requirements of patients with major burns. J Am Diet Assn 65: 415-417, 1974.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A820-00, MILITARY BURN TECHNOLOGY

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN
INJURY IN SOLDIERS -- ABNORMALITIES OF PERIPHERAL
THYROID HORMONE CONCENTRATIONS AND PITUITARY
RESPONSIVENESS IN BURN PATIENTS

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FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

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Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

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Serum thyroid hormone concentrations were measured serially following burn resuscitation in five patients with extensive thermal injuries. Hormone alterations were similar to those observed during other catabolic disease states: serum concentrations of rT_3 increased T_3 decreased, TSH and the free thyroxine index remained within the normal range. The TSH response to TRH was normal in five additional burn patients without bacteremia, was attenuated in four septic patients requiring dopamine infusion, but was hyperresponsive in three relatively hypothermic patients with documented bacteremia not requiring cardiorespiratory support. Although known feedback signals which influence TSH release are altered in the burn patient, the pituitary response to TRH appears preserved until complications and/or treatment disturb this relationship.

Thyroid
 T_3
TRH

THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS
OF BURN INJURY IN SOLDIERS --
ABNORMALITIES OF PERIPHERAL THYROID HORMONE CONCENTRATIONS
AND PITUITARY RESPONSIVENESS IN BURN PATIENTS

Severely burned patients demonstrate many of the clinical features associated with hyperthyroidism such as an elevated basal metabolic rate, tachycardia, hyperventilation, hyperpyrexia, hyperkinesia, and severe weight loss. However, early studies demonstrated normal ^{131}I -uptake and normal serum concentrations of protein bound iodine in thermally injured patients (1), and normal thyroid function in burned animals (2). More recent investigations have related the hypermetabolism following thermal injury to increased sympathetic nervous system activity and not to increased thyroid function (3). However, other alterations in thyroid metabolism have been recently described following a wide variety of stress and catabolic disease states (6-11). Serum concentrations of 3, 5, 3'-triiodothyronine (T_3) decrease, 3, 3', 5'-triiodothyronine (rT_3) increase, and thyroid stimulating hormone (TSH) remains within the normal range. The significance of these alterations is unclear.

-
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 8. Wahl R, Grussendorf M, Magnus J, et al: Changes of thyroid hormone concentration after severe trauma and in hemorrhagic shock. *Eur Surg Res* 9:(suppl 1), 1977.
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 10. Burr WA, Black EG, Griffiths RS, Hoffenberg R, et al: Serum triiodothyronine and reverse triiodothyronine concentrations after surgical operation. *Lancet* 2:1277-1279, 1975.
 11. Burger A, Suter P, Nicod P, et al: Reduced active thyroid hormone levels in acute illness. *Lancet* 1:653-655, 1976.

Because of the severe stress and hypercatabolism associated with burn injury, the present study was designed to assess peripheral thyroid hormone metabolism and pituitary TSH responsiveness to thyrothrin releasing hormone (TRH) at various intervals following thermal injury.

MATERIALS AND METHODS

Sequential measurements of T_3 uptake (T_3U), thyroxine (T_4), free thyroxine index (FTI), T_3 , rT_3 , and TSH were made in five males, mean age 29 years, mean burn size 66.5% (range 53-78%) who were injured simultaneously in a gasoline explosion. Venous blood specimens were obtained at 7:00 A.M., at two to three day intervals beginning on the third post burn day and continuing for the first fifteen days following thermal injury. Serum was immediately separated by centrifugation and the samples frozen until analysis. All but one patient developed bacteremia, demonstrated by positive blood cultures, within the first week of admission, but all patients cleared their sepsis with appropriate antibiotic treatment and were free of systemic infection in the second week of observation.

Twelve additional burn patients (mean age 32 years, mean burn size 62%) were studied a mean of 11 days following injury (range 3-31 days). Four of these critically ill patients required dopamine infusion for cardiocirculatory support following documented bacteremia. In addition, one of the four received exogenous steroids, and all four patients required ventilatory support. The remaining eight patients did not require cardiorespiratory support and did not receive medications known to influence peripheral thyroid hormone concentrations. However, three of these individuals demonstrated positive blood stream cultures for gram negative organisms at the time of study.

Stimulatory studies were performed in the early morning following a six hour fast. Those patients requiring intravenous fluids to maintain hydration received 0.04 molar nutrient-free sodium chloride solutions at a rate predetermined to satisfy fluid requirements and maintain normal hydration. Following placement of a large catheter in a large caliber vein, a period of stabilization was allowed before basal blood samples were drawn for T_4 , T_3 , rT_3 , and serum cortisol measurements. Following this basal sample, TRH, 400 ug, was administered by IV bolus, and blood samples were taken at 15, 30, 45, 60, and 90 minutes following TRH administration. All samples were centrifuged following the test and the serum separated and frozen until analysis.

Serum T_4 was measured by competitive protein binding (4). Previously described radioimmunoassays were employed for the measurement

4. Murphy BE, Patte CJ, Gold A: Clinical evaluation of a new method for the determination of serum thyroxine. J Clin Endocrinol Metab 26:247, 1966.

of TSH, T_3 , and rT_3 (5). Measurements obtained during the different study periods were compared by "t" test analysis of unpaired data.

RESULTS

The sequential studies revealed that the FTI and TSH generally remained within normal limits, while T_3 was reduced and rT_3 elevated (Fig). The T_3U ranged from 35-45%, at or above the normal range (25-35%) while T_4 ranged from 2.5-5.0 ug/dl, with most values generally below the lower limits of normal (4.5 ug/dl).

To identify factors which could differentiate TSH responsiveness to TRH, a profile of patient data and basal concentrations of hormones were compared (Table 1). Serum concentrations of T_4 and T_3 were not significantly different, but cortisol and rT_3 were significantly elevated in the bacteremic patients. Rectal temperature was significantly lower in the bacteremic patients not requiring dopamine.

Following provocative testing with TRH, three types of responses occurred (Table 2). Five patients without complications exhibited a normal increase in TSH concentrations following TRH stimulation, with the peak response averaging 13.8 ± 2.0 uIU/ml. Four critically ill patients receiving dopamine demonstrated an attenuated TSH response to TRH. Finally, three individuals with bacteremia but not requiring cardiorespiratory support, exhibited an accentuated TSH response, the peak TSH value averaging 42.2 ± 6.9 uIU/ml. In addition, the degree of TSH response was generally related to the core temperature of the patients during the test period ($r^2 = 0.51$).

DISCUSSION

These alterations in peripheral thyroid hormonal concentrations are similar to those previously observed in patients with other catabolic disorders. Because these changes may occur with fasting or reduced food intake, the effect of diminished energy intake on these hormonal changes should be separated from the effect of increased sympathetic nervous system activity. In these patients, fluid resuscitation of burn shock is a major priority during the first 48 hours following burn injury. With restoration of blood volume, nutritional intake increases, and high protein-high calorie feedings are instituted. In the patients studied sequentially, weight loss below preinjured body

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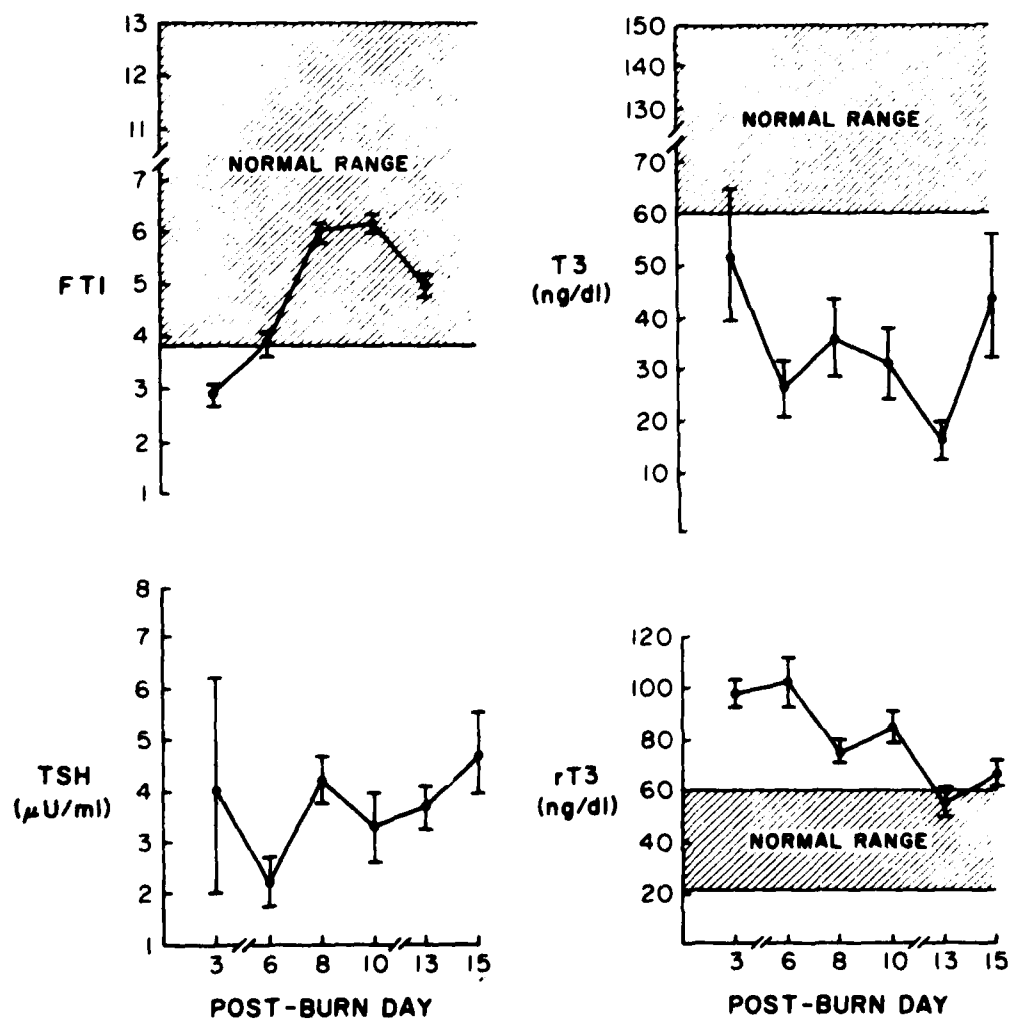


Figure - Serum values (mean \pm S.E.) for T_3 , rT_3 , FTI and TSH in Group I patients. Shaded areas indicate the normal range.

TABLE 1

CHARACTERISTICS OF PATIENTS RECEIVING TRH (MEAN \pm S.E.M. OR RANGE)

Patient Group	n	Age (Years)	Total			Rectal Temperature (°C)	Positive Blood Cultures at time of Study	Basal Hormone Concentrations		
			Body Surface Burn (%)	Postburn Day Studied	Day			T ₄ (ug/dl)	T ₃ (ng/dl)	rT ₃ (ng/dl) Cortisol (ug/dl)
Normal Range								4.5-11.0	80-180	25-65 10-30
Without Complications	5	32 (18-26)	58.5 (38-73)	13 (3-31)		38.6 \pm 0.2	0/5	3.2 \pm 1.0	100 \pm 32	129 \pm 10 31 \pm 5
Bacteremia (Stable)	3	22 (19-28)	62.5 (45-94)	11 (5-16)		37.2 \pm 0.4*	3/3	3.2 \pm 0.5	41 \pm 30	319 \pm 63* 52 \pm 5*
Bacteremia (Dopamine Infusion)	4	39 (15-61)	66.0 (45-79.5)	7 (4-14)		38.4 \pm 0.4	3/4	3.4 \pm 0.5	72 \pm 29	423 \pm 85* 53 \pm 7*

* p<0.01 when compared to uncomplicated patients

TABLE 2
TSH RESPONSE TO TRH IN 12 BURN PATIENTS

Patient Group	n	0	Time in Minutes Following TRH					Peak Response	Δ Response	Integrated Response Above Basal (uIU-min/ml)
			15	30	45	60	90			
Without Complications										
	5	3.6 \pm 1.0	10.3 \pm 1.8	12.1 \pm 1.9	12.3 \pm 1.9	12.9 \pm 2.1	9.3 \pm 1.6	13.8 \pm 2.0	10.2 \pm 1.5	661 \pm 104
Bacteremia										
	3	6.2 \pm 1.4	28.7 \pm 13.5	21.3 \pm 5.2	30.5 \pm 5.8	25.0 \pm 6.1	23.8 \pm 3.2	42.2 \pm 6.9*	36.0 \pm 8.2*	1621 \pm 65**
Bacteremia (Dopamine Infusion)										
	4	2.0 \pm 0.2	2.4 \pm 0.4	3.0 \pm 0.5	3.1 \pm 0.4	2.5 \pm 0.3	3.1 \pm 0.6	1.6 \pm 0.4*	1.6 \pm 0.4*	80 \pm 22*

* $p < 0.01$ when compared to uncomplicated patients

** $p < 0.001$ when compared to uncomplicated patients

weight was minimal, although decreased dietary intake did occur in the early resuscitative phase. The other group of patients received vigorous nutritional support and alterations in thyroid hormone concentrations were observed only with the onset of post-traumatic complications. This would suggest that the dietary influence in these patients did not strongly influence the hormonal alterations observed.

The changes in peripheral thyroid hormone concentrations, associated with the stress of illness, may reflect altered peripheral hormone turnover, changes in pool size, or altered production or release of thyroid hormones. It has been suggested that the degradative mechanism of T_4 is reduced in infectious disease (12, 13). Others have reported that increased turnover of T_3 occurs with certain bacterial infections, although serum concentrations of this hormone may be altered (14). However, recent studies by Eisenstein et al (15) and Suda et al (16) suggest that elevations in serum rT_3 associated with catabolic states are due to a decreased metabolic clearance rate of rT_3 , perhaps reflecting the concomitant fall in serum T_3 . Finally, it has been proposed by Dratman that the T_3 distribution space may be related to the function of the sympathetic nervous system or the functional mass of tissue innervated by the autonomic nervous system (17). T_3 , like its precursors, phenylalanine and tyrosine, may also exhibit increased turnover following severe thermal injury. Such alterations would explain in part the apparent reciprocal relationship between thyroid hormone and catecholamine activity in various disease states as well as thermal injury (18).

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15. Eisenstein A, Hagg S, Vagenakis A, et al: Observations on the peripheral metabolism of 3, 3', 5'-triiodothyronine (reverse T_3 , rT_3) in fed and fasted patients. *Clin Research* 25:294A, 1977.

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An intact hypothalamo-pituitary axis is required for normal thyroid function, and T_3 and T_4 are thought to affect TSH elaboration through feedback mechanisms. Recent studies suggest that glucocorticoids or T_4 , alone, may effectively suppress TSH (19,20). However, TSH responsiveness was normal following TRH administration in the uncomplicated patients, suggesting that neither usual feedback mechanisms nor altered hypo-thalamic function had significantly reduced the pituitary TSH response in those patients. Similarly, intact TSH responsiveness has been observed in patients with infective febrile illnesses (14,21).

Alterations in hypothalamic and pituitary function in extensively injured patients are characterized by an upward shift in central temperature setpoint and a diminished hGH response to provocative stimuli (22). Bacteremia in extensively burned patients is associated with a fall in both metabolic rate and core temperature from predicted values (3), and a decreased rate of gluconeogenesis (23). In contrast to those five patients without bacteremia, alterations in TSH responsiveness occurred in seven bacteremic patients. Four patients receiving dopamine for circulatory support were essentially unresponsive to TRH, a finding consistent with that previously observed during

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dopamine infusion (24, 25). Three additional patients demonstrated an exaggerated TSH response following TRH administration. These patients all demonstrated increased serum concentrations of cortisol and rT_3 and decreased serum T_3 concentrations. In addition, these bacteremic patients with an inadequate febrile response (rectal temperature $< 37.2^{\circ}C$), were relatively hypothermic. Cold stress is reported to increase pituitary release of both ACTH and TSH in animals (26-28). Fisher and Odell have demonstrated significant increments in serum TSH concentrations in human neonates exposed to room temperature for 3-4 hours (29), and Golstein-Golaire et al, have shown an increase of serum TSH concentrations in human adults during and after an acute exposure to cold (30). The TSH hyperresponsive bacteremic patients may have had increased hypothalamic stimulation from temperature or volume receptors resulting in concomitant release of ACTH, reflected by the increased cortisol levels, and an augmented TSH response to TRH.

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25. Burrow GN, May PB, Spaulding SW, et al: TRH and dopamine interactions affecting pituitary hormone secretion. *J Clin Endocrinol Metab* 45:65-72, 1977.

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28. Andersson B: Hypothalamic temperature and thyroid activity. *Ciba Foundation Study Group* 18:35, 1964.

29. Fisher DA, Odell WB: Acute release of thyrotropin in the newborn. *J Clin Invest* 48:1670-1677, 1969.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a		2. DATE OF SUMMARY ^a		3. REPORT CONTROL SYMBOL ^a		
				DA OG 6950		78 10 01		DD FORM 1498, 1 NOV 66		
4. DATE PREV. SUMMARY		5. KIND OF SUMMARY		6. SUMMARY S.C.T. ^a		7. U. N. SECURITY ^a		8. REGRADING ^a		
77 10 01		D. CHANGE		U		U		NA		
9. NO. CODES ^a		10. PROGRAM ELEMENT		11. PROJECT NUMBER		12. TASK AREA NUMBER		13. WORK UNIT NUMBER		
		61101A		3A161101A91C		00		077		
14. PRIMARY		15. CONTRIBUTING		16. CONTRIBUTING						
(U) Hepatic and Muscle Membrane Kinetics in The Endotoxemic Dog: A Preliminary Study For Assessment of Membrane Function in The Septic Thermally Injured Soldier (44)										
2. SCIENTIFIC AND TECHNOLOGICAL AREA ^a 003500 Clinical Medicine										
17. START DATE			18. ESTIMATED COMPLETION DATE			19. FUNDING AGENCY			20. PERFORMANCE METHOD	
76 07			Cont			DA			C. In-House	
21. CONTRACT GRANT Not Applicable										
22. DATES/EFFECTIVE.				23. EXPIRATION:				24. RESOURCES ESTIMATE		
A. NUMBER ^a				B. TYPE:				C. FISCAL YEAR		
				A. AMOUNT:				D. PRECEDING		
A. KIND OF AWARD				F. CUM. AMT.				E. PROFESSIONAL MAN YRS		
								F. FUNDS (In thousands)		
								78 .6 20		
								79 .5 30		
25. RESPONSIBLE DOD ORGANIZATION					26. PERFORMING ORGANIZATION					
NAME: US Army Institute of Surgical Research					NAME: US Army Institute of Surgical Research					
ADDRESS: Ft Sam Houston, Texas 78234					ADDRESS: Ft Sam Houston, Texas 78234					
RESPONSIBLE INDIVIDUAL					PRINCIPAL INVESTIGATOR (Furnish SSAN if U. S. Academic Institution)					
NAME: Basil A. Pruitt, Jr, COL, MC					NAME: Douglas W. Wilmore, MD					
TELEPHONE: 512-221-2720					TELEPHONE: 512-221-4733					
27. GENERAL USE					SOCIAL SECURITY ACCOUNT NUMBER:					
FOREIGN INTELLIGENCE NOT CONSIDERED					ASSOCIATE INVESTIGATORS					
					NAME:					
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28. KEYWORDS (Precede EACH with Security Classification Code)										
(U) Liver; (U) Membrane transport; (U) Sepsis; (U) Indocyanine Green ; (U) Dogs										
29. TECHNICAL OBJECTIVE, 30. APPROACH, 31. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)										
23. (U) To define the alterations in liver function which occur following sepsis by determining hepatic transport characteristics of indocyanine green dye, hepatocyte membrane electrical potential and whole organ metabolic function. Once these abnormalities have been determined, to evaluate the effect of various therapy on these derangements.										
24. (U) Plasma clearance and biliary secretion of indocyanine green dye was determined in 34 dogs before and after endotoxin administration. Hepatocyte membrane potential difference, liver blood flow and hepatic substrate arteriovenous difference was measured in selective experiments. The effect of infusion of amino acids and glucose-insulin was examined as potential treatments for the hepatic derangements.										
25. (U) 7710 - 7809 Endotoxemia in dogs reduced hepatic uptake and biliary excretion of indocyanine green dye. This diminished active membrane transport was associated with reduced hepatocyte membrane potential difference. Studies of arteriovenous concentration differences and flow across the liver demonstrated that endotoxemia increased hepatic glucose and lactate production and decreased oxygen consumption. Correction of this energy deficit occurred following infusion of glucose and insulin, but not after administration of isocaloric quantities of intravenous amino acids.										

^a Available to contractors upon originator's approval.

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PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: HEPATIC AND MUSCLE KINETICS OF THE ENDOTOXEMIC DOG:
A PRELIMINARY STUDY FOR ASSESSMENT OF MEMBRANE FUNCTION
IN THE SEPTIC THERMALLY INJURED SOLDIER -- THE EFFECT
OF EXOGENOUS SUBSTRATE ON HEPATIC METABOLISM AND
MEMBRANE TRANSPORT DURING ENDOTOXEMIA

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

W. Scott McDougal, M.D.
Steven Heimbarger, M.D.
Douglas W. Wilmore, M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: HEPATIC AND MUSCLE KINETICS OF THE ENDOTOXEMIC DOG:
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MEMBRANE TRANSPORT DURING ENDOTOXEMIA

US Army Institute of Surgical Research, Brooke Army Medical Cent.r,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

Investigators: W. Scott McDougal, M.D.
Steven Heimbürger, M.D.
Douglas W. Wilmore, M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Report Control Symbol MEDDH-288(R1)

Endotoxemia in dogs reduced hepatic uptake and biliary excretion of indocyanine green dye. This diminished active membrane transport was associated with reduced hepatocyte membrane potential difference. Studies of arteriovenous concentration differences and flow across the liver demonstrated that endotoxemia increased hepatic glucose and lactate production and decreased oxygen consumption. Correction of this energy deficit occurred following infusion of glucose and insulin, but not after administration of isocaloric quantities of intravenous amino acids. The glucose-insulin infusion during endotoxemia shifted the liver back to an organ of glucose uptake, improved oxygen consumption, and provided the necessary energy for normal dye transport and maintenance of the normal membrane potential difference.

Endotoxin
Hepatic function

THE EFFECT OF EXOGENOUS SUBSTRATE ON HEPATIC METABOLISM AND MEMBRANE TRANSPORT DURING ENDOTOXEMIA

Alterations in hepatic function are central to the compensatory adjustments which occur following severe infection. During septic processes, the liver clears bacterial toxins, processes products of endogenous catabolism, and produces acute-phase proteins, new glucose, and other substrates which are essential for the defense and maintenance of the organism.

Endotoxin impairs the liver's ability to respond to these increased demands by altering hepatocyte function: mitochondrial membrane permeability is increased (7), and active transport of indocyanine green dye (ICG) is diminished (6). However, restoration of normal dye transport occurs when exogenous glucose and insulin are provided. Altered metabolism of endogenous substrate may account for the impaired dye disappearance. Endotoxin causes alterations in hepatic enzyme activity; gluconeogenesis and glycogenesis may be impaired (5), resulting in hepatic glycogen depletion, alterations in blood glucose concentration, and occasional frank hypoglycemia (10). Although the liver plays a central role in the homeostatic response to infection and/or endotoxemia, the definition of the precise nature of altered hepatocyte function during severe infection in vivo is not known and is the purpose of this study.

MATERIALS AND METHODS

Thirty-four mongrel dogs were studied. All animals were vaccinated previously and were free of parasites and other diseases. After a minimum of 10 days of stabilization in our animal facility, 13 animals underwent intravenous sodium methohexital (25 mg/kg of body weight) and Penthrane-oxygen anesthesia, and through a midline

7. Nicholas GG, Mela LM, Miller LD: Early alterations in mitochondrial membrane transport during endotoxemia. *J Surg Res* 16:375, 1974.

6. McDougal WS, Wilmore DW, Pruitt BA Jr: Glucose dependent hepatic membrane transport in nonbacteremic and bacteremic thermally injured patients. *J Surg Res* 22:697, 1977.

5. McCallum RE, Berry LJ: Effects of endotoxin on gluconeogenesis, glycogen synthesis and liver glycogen synthase in mice. *Infect Immunol* 7:642, 1973.

10. Wilmore DW, Mason AD Jr, Pruitt BA Jr: Altered glucose flow in septic burn patients. *Surg Gynecol Obstet* 143:720, 1977.

abdominal incision, two Doppler ultrasonic flow cuffs were placed around the common hepatic artery and portal vein, as described previously (2). The animals were allowed to recover for several weeks before study. After a 12-hour overnight fast, all dogs were anesthetized as described above and maintained with endotracheal tube and esophageal temperature probe in place. Polyvinyl catheters (No. 5) were placed into (1) the left femoral artery, (2) the right femoral artery, (3) the superior vena cava via the external jugular vein, (4) the forelimb peripheral vein, (5) the portal vein via the mesenteric vein, (6) the hepatic vein draining the lateral segment of the left lobe as it joins the hepatic vein draining the middle segment, and (7) the common duct after ligation of the distal common duct and cystic duct. The left femoral artery catheter was attached to a Stratham pressure transducer connected to a Sanborn recorder for continuous monitoring of blood pressure. The superior vena cava catheter was used for a bolus injection of ICG and endotoxin as well as for endotoxin infusions. The peripheral venous route was used for administration of maintenance Ringer's lactate solution and nutrient infusions. Endotoxin and exogenous nutrient infusions were administered in a constant rate by Harvard pump. The right femoral artery, portal vein, hepatic vein, and common bile duct cannulas were used for serial sampling. Hepatic blood flow was determined in the animals with indwelling flow probes as described previously (2).

The animals were allowed to stabilize prior to the beginning of two sequential study periods. At the inception of the first or control period, a bolus of ICG (0.25 mg/kg of body weight) was injected into the superior vena cava. No substrate or endotoxin was administered during this time period. At frequent periodic intervals, bile was collected in preweighed test tubes, and arterial and hepatic venous blood samples were drawn. Thirty minutes after the bolus administration of ICG, blood was collected from the femoral artery, portal vein, and hepatic vein and analyzed for insulin, partial pressure of carbon dioxide, oxygen concentration, hematocrit, hemoglobin, lactic acid, and glucose.

Following the completion of the control study period, the second study period was begun. Twenty-four dogs were given a bolus (0.1 mg/kg of body weight) of *E. coli* endotoxin (*E. coli* 0.26: B6, Difco Laboratories) followed by a constant endotoxin infusion (0.05

2. Heimbürger SL, McDougal WS, Wilmore DW, Pruitt BA Jr: Correction of hepatocellular dysfunction during endotoxemia. *J Surg Res* (in press).

mg/kg·hr). Ten of the 24 animals received Ringer's lactate only, administered at a rate which maintained systemic blood pressure. Nine of the 24 dogs were given by bolus injection 2 gm/kg of 50% dextrose in water and 0.2 unit/kg of regular crystalline insulin, followed by an infusion of glucose (0.5 gm/kg·hr), plus regular insulin (0.2 unit/kg·hr). Five of the 24 dogs received a 2 gm/kg bolus of an 8.5% crystalline amino acid solution (Freamine II), followed by an infusion of amino acid (0.5 gm/kg·hr). All of the study groups received similar intravenous fluid loads.

Ten animals did not receive endotoxin during the second study period to determine the effect of time: four had no substrate infusion during this second control period; three received glucose and insulin as described previously; and three received amino acid infusions. A second bolus of ICG dye was injected at the start of the second period, and blood and bile were collected at similar time intervals, as described for the initial study period.

At the completion of the study, the serum was separated in the blood samples, and both serum and bile were analyzed for ICG concentration, using a Gilford spectrophotometer set at 805 mμ. Serum insulin was determined by the method of Hales and Randle and glucose concentration by the glucose oxidase method. The volume of bile excreted for each time period was determined by weighing the test tubes which contained the bile samples and subtracting the known weight of the empty tube from this value.

Hepatocyte membrane potential differences were measured using glass micropipettes of borosilicate glass pulled to a tip diameter of approximately 0.2 to 0.5 microns and filled by vacuum with a 2M KCl-0.5M KNO₃ solution. Tip resistances were between 4 and 8 megohms. Cork bore biopsies of the liver were obtained in selected animals 30 minutes into each study period, and immediately placed into a Petri dish containing Ringer's lactate. The micropipette was connected via a 2M KCl-0.5M KNO₃ agar salt bridge to a Beckman calomel half-cell reference electrode immersed in Ringer's lactate and then connected to a Keithly electrometer. The circuit was completed via a second reference calomel half-cell immersed in Ringer's lactate and an agar salt bridge, which lay in the Ringer's lactate in a specimen Petri dish. Sequential hepatocyte punctures of the specimen were performed and only those potential differences which were maintained for at least 10 second intervals were recorded.

RESULTS

The disappearance of ICG during the initial control period was similar to previous reports (2) and was unchanged during the sequential control study periods. The concentration of ICG in the hepatic vein always was less than the arterial concentration of simultaneous samples taken during the control studies. The initial rate constant for ICG disappearance was significantly reduced by endotoxin administration (Table I). Hepatic vein dye concentration approached and, in individual studies, exceeded simultaneous arterial concentrations during endotoxemia, reflecting increased back diffusion of ICG from the hepatocyte to the blood (Fig. 1).

Table I. Simultaneous rapid rate constants (k_1) derived from the arterial and hepatic venous disappearance curves for the varying experimental conditions [$-k$, (min^{-1})]

	Control	Endotoxin	Endotoxin-glucose insulin	Endotoxin-amino acids
Artery	0.1026	0.0886	0.1087	0.0842
Hepatic vein	0.1064	0.0720	0.1124	0.0788

Indocyanine green dye disappearance was unaffected by substrate administration during control studies when endotoxin was not administered. The diminished disappearance of ICG during endotoxemia was not observed when glucose and insulin were infused. In contrast, isocaloric infusions of amino acids failed to improve the impaired dye clearance (Table I). The excretion rate of ICG in bile was markedly impaired during endotoxemia (Fig. 2). Glucose and insulin infusion maintained the initial (first 20 minutes) rate of excretion at normal levels, whereas equal calorie infusion of amino acids did not demonstrate this corrective effect.

The hepatocyte membrane potential difference during control periods was -46.4 ± 1.2 mV ($n = 42$), significantly greater ($p < 0.001$) than the membrane potential observed during endotoxin administration (-33.7 ± 1.6) ($n = 20$). The hepatic membrane potential was maintained at $(-53.1 \pm 1.3$ mV) ($n = 31$) when glucose and insulin accompanied the endotoxin infusion. These measurements indicate that effective ion

2. Heimbürger SL, McDougal WS, Wilmore DW, Pruitt BA Jr: Correction of hepatocellular dysfunction during endotoxemia. J Surg Res (in press).

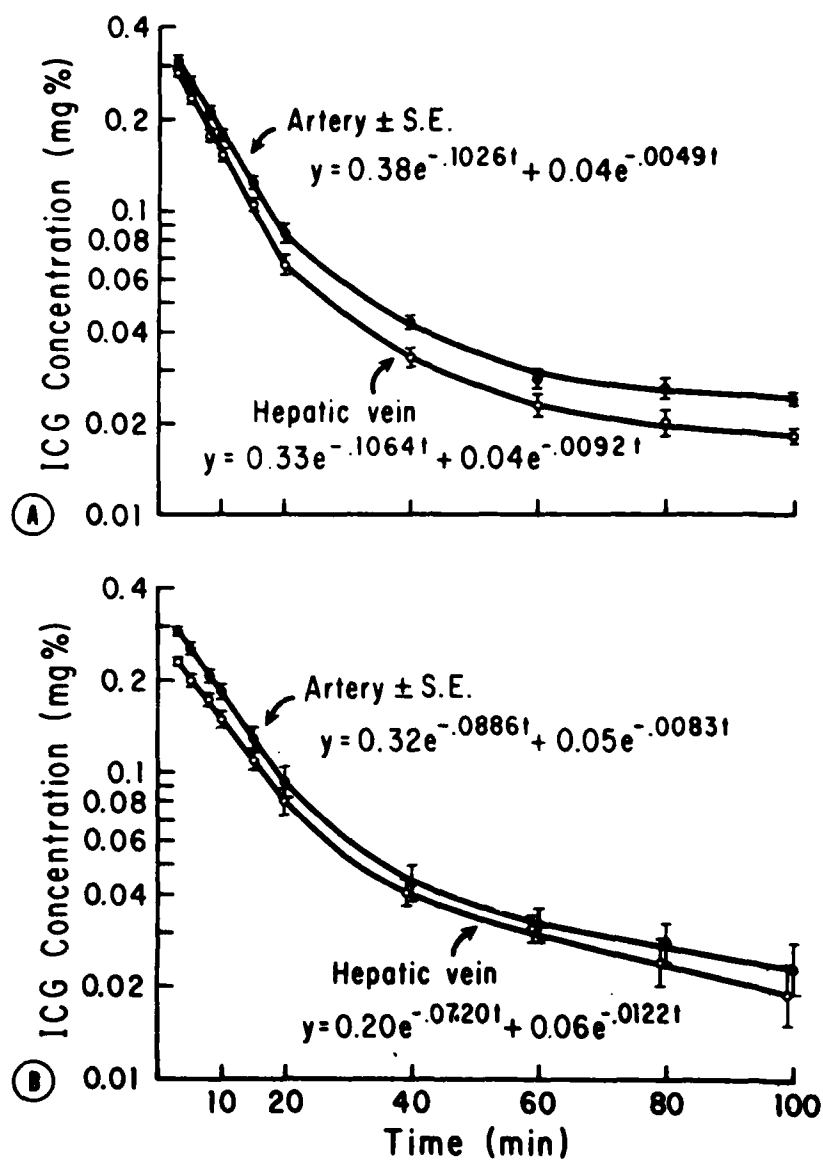


Fig. 1. Composite indocyanine green arterial and hepatic venous plasma disappearance curves for (A) control and (B) endotoxemic animals.

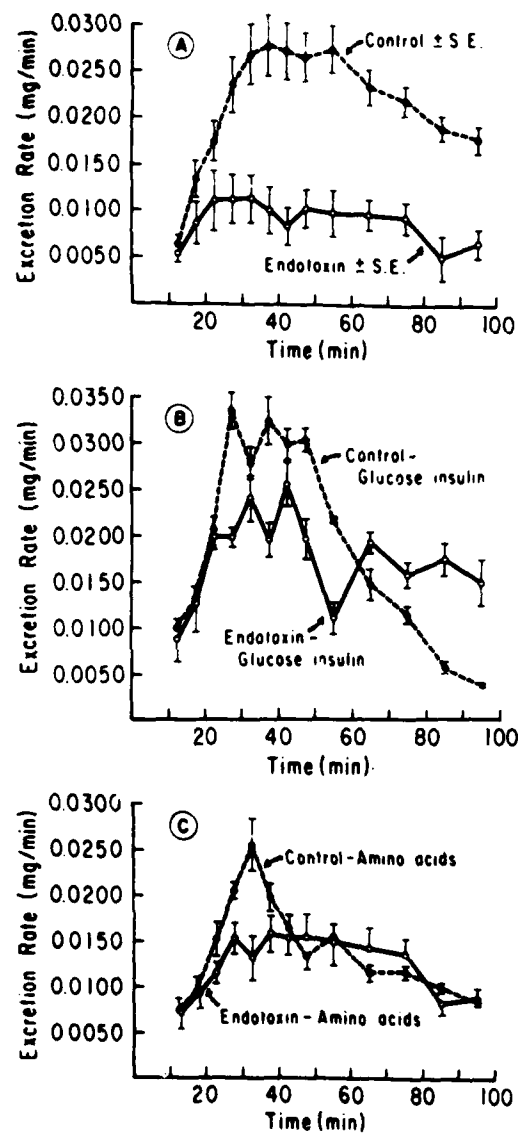


Fig. 2. Average indocyanine green bile excretion rates (milligram per minute \pm standard error) as a function of time for (A) control—endotoxin, (B) control—glucose insulin and endotoxin—glucose insulin, and (C) control—amino acid and endotoxin—amino acid animals.

transport was diminished during endotoxemia, concurrently with diminished active transport of ICG; both were restored to normal levels by the provision of exogenous glucose and insulin.

The mean signal generated by the Doppler ultrasonic flow probes did not change throughout the study periods. Because flow remained constant, alterations in hepatic metabolism were determined by analyzing concentration differences between hepatic inflow and outflow. During endotoxemia, oxygen and carbon dioxide gradients were significantly narrowed, while glucose and lactate increased (Table II). Provision of glucose and insulin returned hepatic oxygen consumption, carbon dioxide production, and net lactate production to normal. During endotoxemia an increase in hepatic glucose occurred, characterized by a widening of the arteriovenous glucose gradient across the liver. Infusion of glucose and insulin during endotoxemia resulted in a net uptake of glucose, when compared with that of the control group, across the liver and a narrowed arteriovenous glucose gradient. This effect was achieved only partially during amino acid infusion. Endogenous arterial insulins were significantly reduced during endotoxemia, falling to $8.8 \pm .8$ uU/ml, as compared with a control value of 14.6 ± 1.0 . Hypoglycemia was not observed during these experiments, and mean blood glucose concentrations for each experimental group of animals ranged between 97 mg/100 ml (a level observed during endotoxemia and amino acid infusion) and 185 mg/100 ml (mean concentrations during glucose-insulin infusion).

DISCUSSION

Endotoxemia results in a reduced rate of active hepatic transport which is reversed with administration of glucose and insulin. This diminished rate of ICG dye transport was observed on both the blood parenchymal and the cannulicular side of the hepatocyte during endotoxin administration. Indocyanine green dye transport is an active process, described by classic enzyme kinetics, and requires two substrates: dye and carbohydrate (6). The provision of glucose, with or without insulin, has been shown in a variety of experimental (2) and clinical studies (6) to be effective in restoring and/or maintaining the organism and the active energy-dependent processes during endotoxemia. Measurements of membrane potential

6. McDougal WS, Wilmore DW, Pruitt BA Jr: Glucose dependent hepatic membrane transport in nonbacteremic and bacteremic thermally injured patients. J Surg Res 22:697, 1977.

2. Heimbürger SL, McDougal WS, Wilmore DW, Pruitt BA Jr: Correction of hepatocellular dysfunction during endotoxemia. J Surg Res (in press).

Table II. Mean arterial concentrations, concentration differences between artery and hepatic vein, and concentration differences between portal vein and hepatic vein for various substances (\pm standard error)*

	Oxygen (ml/100 ml of blood)	Carbon dioxide (mm Hg)	Lactic acid (mg%)	Glucose (mg%)	Insulin (μ U/ml)
<i>Arterial concentration:</i>					
Control	16.7 \pm 0.7	39.4 \pm 3.7	13.9 \pm 1.0	113 \pm 3	14.6 \pm 1.0
Control-glucose insulin	15.3 \pm 0.6	36.3 \pm 8.1	12.9 \pm 0.1	132 \pm 24	296.3 \pm 73.8
Control-amino acids	14.4 \pm 0.9	40.0 \pm 9.2	13.8 \pm 2.8	97 \pm 8	
Endotoxin	17.9 \pm 0.7	38.3 \pm 2.3	17.5 \pm 2.2	106 \pm 8	8.8 \pm 0.8†
Endotoxin-glucose insulin	17.0 \pm 0.9	40.4 \pm 8.0	36.9 \pm 4.4†	185 \pm 49	399.8 \pm 128.0
Endotoxin-amino acids	16.5 \pm 1.1	41.0 \pm 9.8	12.6 \pm 0.8	125 \pm 17	
<i>Artery-hepatic vein:</i>					
Control	4.15 \pm 0.21	-10.9 \pm 1.0	-0.69 \pm 0.48	-7.8 \pm 1.3	-0.6 \pm 1.3
Control-glucose insulin	4.05 \pm 0.20	-11.0 \pm 2.0	0.20 \pm 0.19	-8.8 \pm 1.1	68.0 \pm 44.5
Control-amino acids	4.56 \pm 0.37	-10.0 \pm 1.0	0.97 \pm 0.62	-5.0 \pm 1.8	
Endotoxin†	2.55 \pm 0.30	-8.9 \pm 1.3	-2.00 \pm 0.56	-23.7 \pm 4.1	-3.3 \pm 1.1
Endotoxin-glucose insulin	4.12 \pm 0.16	-11.0 \pm 1.7	1.94 \pm 0.32	-8.0 \pm 1.9	109.3 \pm 32.6
Endotoxin-amino acids†	2.15 \pm 0.36	-9.0 \pm 1.1	-2.16 \pm 0.98	-18.6 \pm 3.7	
<i>Portal vein-hepatic vein:</i>					
Control	1.78 \pm 0.15	-6.0 \pm 2.0	-0.56 \pm 0.48	-16.4 \pm 1.6	6.5 \pm 1.5
Control-glucose insulin	1.65 \pm 0.05	-7.3 \pm 2.1	1.00 \pm 0.30	-5.0 \pm 0.8	99.7 \pm 38.5
Control-amino acids	2.03 \pm 0.51	-6.3 \pm 2.1	0.60 \pm 0.20	-13.7 \pm 1.9	
Endotoxin†	0.81 \pm 0.16	-3.9 \pm 1.0	-3.40 \pm 0.80	-34.1 \pm 6.3	3.1 \pm 1.1
Endotoxin-glucose insulin	1.96 \pm 0.20	-8.2 \pm 1.3	1.64 \pm 0.87	-6.9 \pm 1.8	185.3 \pm 49.2
Endotoxin-amino acids†	1.06 \pm 0.07	-4.8 \pm 2.9	-1.28 \pm 0.49	-20.2 \pm 3.3	

*Positive integers indicate hepatic uptake and negative integers indicate hepatic release.

†Values and groups which are significantly different ($p < 0.01$) from controls.

difference which reflect the integrity of the ion pump and/or hepatocyte membrane permeability confirm that derangements of cell energetics occur at the membrane level. The fall in potential difference which occurs during endotoxemia can be restored to normal by infusion of glucose and insulin.

The permeability of the hepatocyte membrane also is increased during endotoxin infusion. Immediately following the administration of ICG, hepatocyte concentration is low and blood concentration high. Diffusion from blood into hepatocyte is favored. However, as the blood concentration falls and the intracellular dye concentration increases due to the active transport of the ICG into the cell, diffusion from hepatocyte to blood is favored. Thus if permeability remains constant and is relatively low for the dye, the rate of disappearance of dye from the hepatic vein should parallel or exceed the rate of disappearance of dye from the arterial circulation at all plasma concentration levels. Under control conditions this was found to be the case (Table I), indicating that membrane permeability to ICG was minimal. However, when endotoxin was administered, the rate of disappearance from the hepatic vein was less than the rate of disappearance from the arterial circulation (Table I), and, at times in individual experiments, the venous dye concentrations exceeded arterial levels. This indicates that membrane permeability was increased and that back diffusion of dye was significant when hepatocyte concentration was high and blood concentration low.

Previous studies have characterized the direct cellular effects of endotoxin on cellular energy mechanisms (7). Associated with decreases in hepatocyte concentrations of adenosine triphosphate (ATP) is marked depletion of liver glycogen. Impaired glycogen synthase activity and impaired incorporation of alanine and pyruvate into new glucose are known effects of endotoxin (1,5). In these experiments a marked increase in hepatic glucose release was observed in response to endotoxin infusion, consistent with glycogenolysis which is an early and common hepatic metabolic response during stress.

7. Nicholas GF, Mela LM, Miller LD: Early alterations in mitochondrial membrane transport during endotoxemia. J Surg Res 16: 375, 1974.

1. Hamosh M, Shapiro B: Mechanism of glycogenolytic action of endotoxin. Br J Exp Pathol 41:372, 1960.

5. McCallum RE, Berry LJ: Effects of endotoxin on gluconeogenesis, glycogen synthesis and liver glycogen synthase in mice. Infect Immunol 7:642, 1973.

Not only are glycogen stores depleted by endotoxin, but hepatic oxidative energy metabolism appears to be impaired. Endotoxemia results in diminished hepatic oxygen consumption, decreased carbon dioxide production, and increased lactic acid generation, indicating a decrease in aerobic metabolism and a shift to anaerobic mechanisms. This finding is consistent with in vitro studies demonstrating endotoxin inhibition of succinate oxidation at the substrate end of the respiratory chain (7). Thus endotoxin limits the tricarboxylic acid cycle energy production and shifts metabolism toward anaerobic glycolysis. This limitation in energy availability is expressed in decreased cell function, i.e., impaired active transport on both the blood and cannicular sides of the hepatocyte and a fall in potential difference across the cell membrane.

But why should the glucose-insulin infusion return these functions to normal? It could be argued that glucose exerts an osmotic effect which somehow prevents endotoxin-induced periportal edema which would impair transport of oxygen. However, the glucose-insulin solution did not greatly increase serum osmolality (plasma glucose rose only from a mean of 97 to 185 mg/100 ml), and calculated serum osmolality did not account for major osmotic changes during the infusion. Moreover, the amino acid infusion also should exert an osmotic effect, but similar improvement in hepatocyte function was not observed.

However, the studies of arteriovenous differences demonstrate that hepatic uptake of both glucose and insulin occurs during the infusion of this mixture. It has been suggested that glucose and/or insulin plays a specific role in the maintenance of the integrity of the hepatocyte. Glucose and/or insulin has been related to the energy charge of the hepatocyte (3) and insulin has been proposed as the primary factor controlling alterations in hepatocyte mitochondrial metabolism during liver regeneration. Phosphorylative activity is restored to normal in the ischemic liver following insulin administration, and the hepatocyte turnover during liver regeneration increases markedly with chronic infusion of insulin

7. Nicholas GG, Mela LM, Miller LD: Early alterations in mitochondrial membrane transport during endotoxemia. *J Surg Res* 16: 375, 1974.

3. Kimura K, Kamiyama Y, Ozawa K, Honjo I: Changes in adenylate energy charge of the liver after oral glucose load. *Gastroenterology* 70:665, 1976.

into an isolated hepatic lobe (8). In a similar manner, the hepatic impairment observed with endotoxemia may be ameliorated or reversed by the glucose-insulin environment. Amino acids do not exert comparable effects because of their decreased insulinogenic capacity, lack of ability to support glycolytic metabolic pathways when compared to glucose, or the specific intracellular competition for energy which occurs with conversion of nitrogen to urea.

Finally, these experimental observations should not be over-interpreted in the care of critically ill septic patients. These findings and those of others may help to explain in part the pathogenesis of the hepatic dysfunction and cholestatic jaundice which frequently is associated with bacteremia or endotoxemia (9). Moreover, the studies lend some scientific basis to the previously held concepts that hepatic glycogen stores or glucose infusions protect or support liver function during a variety of clinical infections (4). However, this information should not be interpreted to suggest that glucose-insulin therapy be utilized routinely in all critically ill bacteremic patients. Rather, as is present clinical practice, complete and balanced nutritional support which contains a high portion of carbohydrate calories and is administered by the enteral route, if possible, remains the best method of metabolic support until the septic focus can be eliminated and the bacteria/endotoxin is cleared from the blood stream.

8. Starzel TE, Porter KA, Watanabe K, Putnam CW: Effects of insulin, glucagon, and insulin/glucagon infusions on liver morphology and cell division after complete portacaval shunt in dogs. *Lancet* 1:821, 1976.

9. Utili R, Abernathy CO, Zimmerman HJ: Studies on the effects on *E. coli* endotoxin on cannicular bile formation in the isolated perfused rat liver. *J Lab Clin Med* 89:471, 1977.

4. Martin E: Dextrose therapy in everyday practice, New York, 1937, Paul B. Hoeber, Inc., Medical Book Division of Harper & Row, Publishers.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1 AGENCY A. LESSON ¹		2 DATE OF SUMMARY ²		REPORT CONTROL SYMBOL DD FORM 1498A, 1 MAR 68	
3 DATE PREV SUMMARY ³		4 KIND OF SUMMARY ⁴		5 SUMMARY ACT ⁵		6 WORK SECURITY ⁶		7 REGRADING ⁷	
77 10 01		D. CHANGE		U		U		NA NL	
10 NO. CODES ¹⁰		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY		61101A		3A161101A91C		00		089	
B. CONTRIBUTING									
C. CONTRIBUTING									
11 TITLE (Precede with Security Classification Code) ¹¹ (U) Use of a Laminar Flow Isolator to Control Infection in Burned Troops (44)									
12 SCIENTIFIC AND TECHNOLOGICAL AREAS ¹² 003500 Clinical Medicine									
13 START DATE			14 ESTIMATED COMPLETION DATE			15 FUNDING AGENCY		16 PERFORMANCE METHOD	
77 09			Cont			DA		C. In-House	
17 CONTRACT GRANT Not Applicable				18 RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS		B. FUNDS (In thousands)	
A. DATES/EFFECTIVE:				EXPIRATION:		FISCAL YEAR		C. CURRENT	
B. NUMBER ¹⁸						78		.3	
C. TYPE:				D. AMOUNT:		79		.4	
E. KIND OF AWARD				F. CUM. AMT.				25	
19 RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION					
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research					
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TELEPHONE: 512-221-2720				TELEPHONE: 512-221-3301					
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:					
FOREIGN INTELLIGENCE NOT CONSIDERED				ASSOCIATE INVESTIGATORS					
				NAME:					
				NAME: DA					
22 KEYWORDS (Precede EACH with Security Classification Code)									
(U) Burn injury; (U) Infection; (U) Laminar flow; (U) Humans									
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)									
23. (U) It has been well known in recent years that the development of infection has been the most common cause of death in burned soldiers. As the vast majority of these cases result from invasive infection of the burn wound, methods of reducing burn wound contamination would be expected to result in improved survival. In addition, studies have shown that cross-contamination colonization causes more invasive burn wound infections than auto-contamination colonization. These facts generated interest in the use of laminar air flow isolator units as part of burn care.									
24. (U) The Sci-Med Company of Minneapolis, Minnesota, was contracted to develop a Laminar air flow unit to meet certain specifications. Following initial temporary installation, initial testing, and initial patient trials, numerous modifications and new developments were thought to be necessary and were undertaken. These have been completed, and the unit is now installed in its permanent form. Comparison of burn wound microbiology between laminar flow and conventionally treated patients will be made and related to septic complications and mortality in both groups.									
25. (U) 7710 - 7809 Initial testing of the air filtration system is on-going, and simultaneous samplings of air within the isolator unit and air in our intensive care section are being evaluated. It has been determined that adult male patients with a wide range of burn injuries can successfully receive treatment while in the laminar air flow isolator unit, with some exceptions in those patients requiring extensive medical treatment. Numerous modifications in nursing techniques are currently being devised to cope with special problems which were recognized in the early treatment of patients in the laminar air flow isolator unit. Once the initial environmental microbiological studies and development and modifications of nursing techniques have been completed, patient care trials will be undertaken.									

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: USE OF A LAMINAR FLOW ISOLATOR TO CONTROL INFECTION IN
BURNED TROOPS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

William F. McManus, M.D., Lieutenant Colonel, MC
Richard C. Treat, MD, Major, MC
Robert B. Lindberg, Ph.D.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: USE OF A LAMINAR FLOW ISOLATOR TO CONTROL INFECTION IN
BURNED TROOPS

US Army Institute of Surgical Research, Brooke Army Medical Center
Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

Investigators: William F. McManus, M.D., Lieutenant Colonel, MC
Richard C. Treat, M.D., Major, MC
Robert B. Lindberg, Ph.D.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

The effectiveness of a laminar flow isolator unit to protect the burn patient from bacterial colonization is currently under evaluation. Comparison of patients treated for ten days within the laminar flow isolator unit with those patients managed with routine aseptic precaution in beds adjacent to the laminar flow unit will determine whether there is any significant advantage to the burn patient from such a protected environment.

Burn injury
Infection
Laminar flow
Humans

USE OF A LAMINAR FLOW ISOLATOR TO CONTROL INFECTION IN BURNED TROOPS

This study is designed to evaluate the effectiveness of the laminar flow isolator unit for the protection of the burn patient from bacterial contamination. Patients admitted to the Institute of Surgical Research within 24 hours post burn and with a total body surface injury exceeding 40% are considered for placement in the prepared laminar flow unit. On admission the patient has blood, wound and tracheal aspirate cultures and subsequent blood and wound cultures daily for 10 days. Tracheal aspirate cultures are obtained as indicated. The patients are removed from the laminar flow unit for routine wound care or operation as indicated. The length of study in the laminar flow unit is 10 days. The marker is the Staphylococcal aureus phage type 84 of characteristic antibiogram. The control for this group of patients are patients in adjacent beds in the Intensive Care Unit who have similar wound, blood, and tracheal aspirate cultures. Utilizing the ubiquitous Staphylococcus aureus which has commonly appeared in patients with over 40% total body surface injury as early as three to five days post burn will make it possible to conclude whether a laminar flow unit delays colonization. If a significant delay in colonization is identified a second group of patients undergoing similar wound management will be examined in the laminar flow bed with the hepa filters removed, to determine whether delay in colonization is a laminar flow effect or an effect of improved technique.

If no difference in colonization rates are noted, an additional group of patients will be studied for 10 days without the patients being removed from the laminar flow isolator for bathing and wound care.

This study as of the date of this report is ongoing.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a		2. DATE OF SUMMARY ^a		REPORT CONTROL SYMBOL	
				DA OG 6954		78 10 01		DD-DR&E/AR 1036	
3. DATE PREL. SUMMARY ^a		4. KIND OF SUMMARY		5. SUMMARY SCTY ^a		6. WORK SECURITY ^a		7. REGRADING ^a	
77 10 01		H. TERM.		U		U		NA	
10. NO. CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
		61101A		3A161101A91C		00		076	
8. PRIMARY		61101A		3A161101A91C		00		076	
9. CONTRIBUTING									
11. CONTRIBUTING									
11. TITLE (Precede with Security Classification Code) ^a (U) Effect of Cyclic Nucleotides on Proximal Tubular Reabsorption as an Influence on Renal Dysfunction in Injured Soldiers (44)									
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a									
003500 Clinical Medicine									
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17. CONTRACT GRANT									
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18. DATES/EFFECTIVE				19. EXPIRATION				20. RESOURCES ESTIMATE	
A. NUMBER ^a				B. TYPE				C. AMOUNT	
A. KIND OF AWARD				F. CUM. AMT.				D. FUNDS (in thousands)	
								10	
21. RESPONSIBLE DOD ORGANIZATION					22. PERFORMING ORGANIZATION				
NAME ^a US Army Institute of Surgical Research					NAME ^a US Army Institute of Surgical Research				
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TELEPHONE 512-221-2720					TELEPHONE 512-221-4264				
23. GENERAL USE					24. ASSOCIATE INVESTIGATORS				
FOREIGN INTELLIGENCE NOT CONSIDERED					NAME:				
					NAME:				
25. KEYWORDS (Precede EACH with Security Classification Code)									
(U) Cyclic nucleotides; (U) Proximal convoluted tubule; (U) In-vitro microperfusion									
26. TECHNICAL OBJECTIVE, 27. APPROACH, 28. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)									
<p>23. (U) The present project is designed to examine the role of cyclic nucleotides in fluid reabsorption in the proximal convoluted tubule of the kidney. Cyclic AMP may be an intermediate messenger by which various hormones (parathormone) and vasoactive substances (angiotensin, epinephrine) express their effects on proximal tubular reabsorption. It is felt that the results of this project might help us to understand the pathophysiology of altered renal function in burned patients.</p> <p>24. (U) All experiments will be conducted utilizing the technique of <u>in vitro</u> microperfusion of proximal convoluted tubules dissected from rabbit kidneys. Tubules are dissected, transferred to a perfusion chamber kept at 37°C and pH 7.4, hooked up to specially constructed pipets where they are perfused with an ultrafiltrate of serum and bathed in serum.</p> <p>25. (U) 77 10 - 78 09 This project has been completed. These studies show that cyclic nucleotides decrease reabsorption of salt and water in the proximal tubule of the kidney by changing the permeability of the tubular epithelium. The results of these experiments have been presented at the 1977 meeting of the American Federation of Clinical Research. In addition, a manuscript entitled, "The Role of Altered Permeability in The Proximal Tubule Response to Cyclic AMP" has been accepted for publication in the American Journal of Physiology.</p>									

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TERMINATION

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT
RESEARCH

REPORT TITLE: EFFECT OF CYCLIC NUCLEOTIDES ON PROXIMAL
TUBULAR REABSORPTION AS AN INFLUENCE ON
RENAL DYSFUNCTION IN INJURED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigator

Harry R. Jacobson, M.D., Major, MC

Reports Control Symbol MEDDH-288 (RI)

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ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: Effect of Cyclic Nucleotides on Proximal Tubular Reabsorption as an Influence on Renal Dysfunction in Injured Soldiers

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

Investigator: Harry R. Jacobson, M.D., Major, MC

Reports Control Symbol MEDDH-288 (RI)

The microperfusion laboratory of the Institute of Surgical Research has been involved in studies of renal tubules perfused *in vitro*. These studies have centered around the permeability properties of proximal convoluted tubules (PCT) and how these properties are related to volume reabsorption. Two projects have been completed.

First, the effects of cyclic AMP on PCTs were examined. Superficial PCTs dissected from rabbit kidneys were perfused *in vitro* with an ultrafiltrate of rabbit serum and bathed in rabbit serum. Transepithelial potential difference (PD), net fluid reabsorption (Jv) and bath to lumen sucrose permeability were measured. Sucrose permeability was used as an index of the permeability of intercellular spaces.

An analogue of cyclic AMP (8 chloro phenylthio cAMP) was shown to: (1) reversibly decrease the lumen negative PD; (2) reversibly inhibit net fluid reabsorption; and (3) increase the bath to lumen sucrose permeability. Similar studies utilizing the active transport inhibitor, ouabain, resulted in greater inhibition of PD and Jv but no significant change in sucrose permeability. It was concluded from these studies that cyclic nucleotides inhibit proximal tubule volume reabsorption by altering the permeability of the paracellular pathway and allowing backleak of outwardly transported solute.

An abstract of this work was presented at the American Federation for Clinical Research in Washington, D.C. May, 1977. In addition, a manuscript entitled "The role of altered permeability in the proximal tubule response to cyclic AMP" has been accepted for publication in The American Journal of Physiology.

The second group of microperfusion experiments were concerned with comparing reabsorptive characteristics of proximal tubules from superficial and juxtamedullary nephrons. Proximal convoluted tubule segments from each nephron population were perfused with various solutions designed to examine fluid reabsorptive rates and transepithelial potentials when: (1)

(1) perfusion fluid simulated normal glomerular filtrate; (2) perfusion fluid contained a higher (Cl) and lower (HCO_3) than bath fluid (simulating in vivo conditions in the later proximal tubule),³ (3) perfusion fluid lacked solutes normally considered to support active Na reabsorption (HCO_3 , glucose, amino acids).

These studies have shown significant intrinsic differences between superficial and juxtamedullary convolutions. While both segments have similar reabsorptive rates when perfused with fluid simulating glomerular filtrate, when perfused with high (Cl) low (HCO_3) fluid the superficial tubule accomplished reabsorption mostly via passive mechanisms while the juxtamedullary tubule appears to lack this capability.

It was concluded that the superficial and juxtamedullary proximal convolutions, which have previously been shown to have different relative Na and Cl permeabilities, also accomplish fluid reabsorption via different mechanisms. The superficial segment, because of its higher Cl permeability exhibits its passive volume reabsorption linked to the normally generated lumen to blood Cl gradient. The juxtamedullary segment, which has a higher relative Na permeability does not exhibit a significant volume flux linked to Cl gradients.

An abstract of this work was presented at the American Society of Nephrology in Washington, D.C. in November, 1977. Also, a manuscript entitled "Characteristics of Volume Reabsorption in Rabbit Superficial and Juxtamedullary Proximal Convolutions" has been submitted to the Journal of Clinical Investigation.

Cyclic nucleotides
Proximal convoluted tubule
In vitro microperfusion

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a		2. DATE OF SUMMARY ^a		REPORT CONTROL SYMBOL DD DR&E(AR)636	
3. DATE PREV SUMMARY ^a		4. KIND OF SUMMARY ^a		5. SUMMARY SCTY ^a		6. WORK SECURITY ^a		7. REGRADING ^a	
77 10 01		K. COMP.		U		U		NA	
8. DDD'S INSTN ^a		9. SPECIFIC DATA CONTRACTOR ACCESS		10. LEVEL OF SUM A. WORK UNIT					
NL		<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO							
10. NO / CODES ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY		61101A		3A161101A91C		00		084	
B. CONTRIBUTING									
C. CONTRIBUTING									
11. TITLE (Precede with Security Classification Code) ^a (U) Echocardiographic Evaluation of Left Ventricular Performance in The Severely Burned Military Population (44)									
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine									
13. START DATE			14. ESTIMATED COMPLETION DATE			15. FUNDING AGENCY		16. PERFORMANCE METHOD	
76 06			78 09			DA		C. In-House	
17. CONTRACT/GRANT Not Applicable									
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A. KIND OF AWARD			F. CUM. AMT.			CURRENT			
19. RESPONSIBLE DOD ORGANIZATION					20. PERFORMING ORGANIZATION				
NAME ^a US Army Institute of Surgical Research					NAME ^a US Army Institute of Surgical Research				
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21. GENERAL USE					SOCIAL SECURITY ACCOUNT NUMBER:				
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22. KEYWORDS (Precede EACH with Security Classification Code) (U) Postburn shock; (U) Echocardiography; (U) Humans (U) Resuscitation; (U) Burned soldiers; (U) Left ventricular function; (U) Cardiac output									
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)									
23. (U) To evaluate left ventricular function in thermally injured soldiers, especially in the postburn shock phase. To evaluate the hypothesis that myocardial depression is a direct consequence of severe thermal injury.									
24. (U) Serial left ventricular performance profiles will be derived echocardiographically and correlated to the clinical state of the patient.									
25. (U) 77 10 - 78 09 Serial echocardiographic (ECHO) evaluation of left ventricular function has been performed in 45 thermally injured patients (135 total over a two-year period). At the termination of the study, it is concluded that (1) ECHO is a valuable noninvasive diagnostic adjunct in the critical care unit; (2) ECHO can be performed serially following severe thermal injury to assess the efficacy of therapy; (3) ECHO can also be performed during acute respiratory failure and its treatment with continuous positive pressure ventilation; and (4) left ventricular performance can be serially evaluated during various "shock" syndromes treated in a critical care unit.									

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TERMINATION

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR PERFORMANCE IN THE SEVERELY BURNED MILITARY POPULATION

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

James F. Dorethy, M.D., Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR PERFORMANCE IN THE SEVERELY BURNED MILITARY POPULATION

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

Investigators: James F. Dorethy, M.D., Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Echocardiography (ECHO) was utilized to monitor left ventricular (LV) performance after acute thermal injury and its complications. Serial ECHO was performed in 135 thermally injured patients over a two-year period. At the termination of the study, it is concluded that ECHO is a valuable noninvasive diagnostic adjunct in the critical care unit. It offers the advantage of a direct measurement of myocardial contractility and an estimation of intravascular volume. This study also correlated ECHO measurements with standard critical care invasive monitoring. In 80 studies, the correlation coefficient of LV end-diastolic volume (EDV) to pulmonary artery wedge (PAW) pressure was $r = .76$. Echocardiographic cardiac output was closely correlated with cardiac output obtained by thermal dilution ($r = .97$), with an apparent ECHO underestimation of 1.8 L/min. Echocardiographic ejection fraction did not correlate with either PAW or EDV ($r = .03$ and $.30$ respectively). Therefore, ECHO-EDV accurately reflected indirectly measured LV filling pressure (PAW). Ejection fraction was not dependent on any of the variables tested. Although this phase of the study has been concluded, other protocols have been established to evaluate LV function in specific postburn clinical situations.

Postburn shock
Echocardiography
Resuscitation
Left ventricular function
Cardiac output
Burned soldiers

ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR PERFORMANCE IN THE SEVERELY BURNED MILITARY POPULATION

Monitoring the cardiovascular response to acute thermal injury, postburn resuscitation, and subsequent complications is difficult and often requires invasive techniques. A promising noninvasive technique to evaluate left ventricular (LV) performance in these situations is echocardiography (ECHO). ECHO LV ejection indices and volumes compare favorably with those obtained by angiographic contrast studies (1-4). It has also been of diagnostic usefulness in pericardial effusion and tamponade (5-6), bacterial endocarditis (7), hypertrophic obstructive cardiomyopathy (8-9), rheumatic valvular disease (10), and some aspects of ischemic heart disease (11).

1. Fortuin NJ, Hood WP Jr, Craige E: Evaluation of left ventricular function by echocardiography. *Circulation* 46:26-35, 1972.
2. Cooper RH, O'Rourke RA, Karliner JS, Peterson KL, Leopold GR: Comparison of ultrasound and cineangiographic measurements of the mean rate of circumferential fiber shortening in man. *Circulation* 46:914-923, 1972.
3. Ludbrook P, Karliner JS, Peterson K, Leopold G, O'Rourke RA: Comparison of ultrasound and cineangiographic measurements of left ventricular performance in patients with and without wall motion abnormalities. *Br Heart J* 35:1026-1032, 1973.
4. Teichholz LE, Kreulen T, Herman MV, Gorlin R: Problems in echocardiographic volume determinations: Echocardiographic-angiographic correlations in the presence or absence of asynergy. *Am J Cardiol* 37:7-11, 1976.
5. Nanda NC, Gramiak R, Gross CM: Echocardiography of cardiac valves in pericardial effusion. *Circulation* 54:500-504, 1976.
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7. Lee C-C, Ganguly SN, Magnisalis K, Robin E: Detection of tricuspid valve vegetations by echocardiography. *Chest* 66:432-433, 1974.
8. Popp RL, Harrison DC: Ultrasound in the diagnosis and evaluation of therapy of idiopathic hypertrophic subaortic stenosis. *Circulation* 40:905-914, 1969.
9. Henry WL, Clark CE, Epstein SE: Asymmetric septal hypertrophy: Echocardiographic identification of the pathognomonic anatomic abnormality of IHSS. *Circulation* 47:225-233, 1973.
10. Popp RL: Echocardiographic assessment of cardiac disease. *Circulation* 54:538-552, 1976.
11. Stack RS, Lee CC, Reddy BP, Taylor ML, Weissler AM: Left ventricular performance in coronary artery disease evaluated with systolic time intervals and echocardiography. *Am J Cardiol* 37:331-344, 1976.

Serial measurements of acute LV dimensional alterations during heart rate change (12), afterload alteration (12), intravascular volume change (13), and positive or negative inotropic intervention (14-15) have been reported. Echocardiographic studies on normal populations have established quantitative and qualitative guidelines (16-17). Autopsy comparison of LV and septal wall size reveals close correlation (18). The limitations and resolution capabilities of single mode ECHO scanning are well known (19-21).

This study evaluated ECHO LV performance during postburn hospitalization. An earlier report (22) summarized the initial findings

12. Hirshleifer J, Crawford M, O'Rourke RA, Karliner JS: Influence of acute alterations in heart rate and systemic arterial pressure on echocardiographic measures of left ventricular performance in normal human subjects. *Circulation* 52:835-841, 1975.
13. Redwood DR, Henry WL, Epstein SE: Evaluation of the ability of echocardiography to measure acute alterations in left ventricular volume. *Circulation* 50:901-904, 1974.
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15. Frishman W, Smithen C, Befler B, Kligfield P, Killip T: Noninvasive assessment of clinical response to oral propranolol therapy. *Am J Cardiol* 35:635-644, 1975.
16. Quinones MA, Gaasch WH, Alexander JK: Echocardiographic assessment of left ventricular function: with special reference to normalized velocities. *Circulation* 50:42-51, 1974.
17. Tynan M, Reid DS, Hunter S, Kaye HH, Osme S, Urquhart W, Davies P: Ejection phase indices of left ventricular performance in infants, children, and adults. *Br Heart J* 37:196-202, 1975.
18. Maron BJ, Henry WL, Roberts WC, Epstein SE: Comparison of echocardiographic and necropsy measurements of ventricular wall thicknesses in patients with and without disproportionate septal thickening. *Circulation* 55:341-346, 1977.
19. Roelandt J, van Dorp WG, Bom N, Laird JD, Hugenholtz PG: Resolution problems in echocardiography: a source of interpretation errors. *Am J Cardiol* 37:256-262, 1976.
20. Linhart JW, Mintz GS, Segal BL, Kawai N, Kotler MN: Left ventricular volume measurement by echocardiography: Fact or fiction? *Am J Cardiol* 36:114-118, 1975.
21. Karliner JS, O'Rourke RA: Usefulness and limitations of assessment of internal shortening velocity by ultrasound in man. *Chest* 68:361-364, 1975.
22. Dorethy JF: Echocardiographic evaluation of left ventricular performance in the severely burned military population. Annual Research Progress Report, US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas, 30 September 1977, pp 388-406.

utilizing this technique. This study concludes the project and reports on the efficacy of ECHO to estimate various hemodynamic measurements obtained by invasive methods.

METHODS AND MATERIALS

Technique

All studies were performed with a specially designed mobile Honeywell ECHO/hemodynamic unit interfaced with a Honeywell 1858 Visicorder and Hewlett-Packard 1317A display scope. M-mode echograms were obtained with a commercially available Ekoline 20A ultrasonoscope. Transducers included 3.5 and 2.25 MHz sizes focused at 5, 7.5 and 10 cm. Transducers of higher frequency and variable focal distance were used whenever possible to facilitate the recording of detailed ECHO anatomy.

All studies were performed with minimal interruption of routine and emergency care. Patients were studied initially, and then serially as their clinical course dictated. All were studied supine, semi-erect, or in a semi-left lateral position. The patient position, transducer angulation and standard interspace were carefully noted. Standard interspace was defined as the transducer being perpendicular to the chest wall with visualization of the anterior mitral leaflet (23). The external relationship of the transducer to the heart was duplicated as closely as possible in serial studies. Only qualitative ECHO findings were used in those patients without adequate ECHO "windows" required for an M-mode sector scan. No attempt was made to establish the efficacy of the technique in every patient. Only those with adequate echograms as defined above were included in serial follow-up.

The ECHO measurements used to evaluate LV performance are listed in Table 1. The methodology was identical to that from the earlier report (22).

All values were compared to a normal ECHO population. These were patients who had normal right and left heart catheterizations (courtesy of Brooke Army Medical Center, Cardiac Catheterization Laboratory, LTC Joseph P. Murgo, M.D., Chief). These normal values and their corresponding angiographic measurements are listed in Table 2. Statistical analysis was performed using the Student's t-test for unpaired data.

23. Popp RL, Filly K, Brown OR, Harrison DC: Effect of transducer placement on echocardiographic measurement of left ventricular dimensions. *Am J Cardiol* 35:537-540, 1975.

Table 1. Echocardiographic Measurements
of Left Ventricular Function

1. LV end-diastolic volume (cc) = EDV
2. LV end-diastolic volume index (cc/m²) = EDVI
3. LV end-systolic volume (cc) = ESV
4. LV end-systolic volume index (cc/m²) = ESVI
5. Stroke volume (cc/bt) = SV
6. Stroke index (cc/bt/m²) = SI
7. ECHO-cardiac output (L/min) = CO
8. ECHO-cardiac index (L/min/m²) = CI
9. Ejection fraction (%) = EF
10. Mean velocity of diameter shortening
(circ/sec) = V_{cf}

Table 2. Normal ECHO Left Ventricular Indices
Compared to Angiographic Indices

n = 25			Age = 38 ± 7			BSA = 1.86 ± 0.19		
ECHO			ANGIO					
EDV	111 ± 32	(59 ± 15)	121 ± 30	(64 ± 14)				
ESV	30 ± 12	(16 ± 6)	34 ± 14	(18 ± 7)				
SV	81 ± 21	(43 ± 10)	87 ± 22	(46 ± 10)				
EF	0.74 ± 0.06	---	0.72 ± 0.08	---				
V _{cf}	1.22 ± 0.22	---	1.44 ± 0.34	---				

Values = mean ± 1 SD; abbreviations and units same as Table 1; ANGIO = cineangiographic measurements from LAO position; () = calculated "index" per m².

Right-sided pressures were obtained via a commercially available balloon-directed Instruments Laboratory (IL) or Edwards catheter. Left-sided pressures were obtained from the femoral artery with standard intracatheter setups. Left ventricular cavity pressure was measured by a specially designed 110 cm polyethylene catheter inserted retrograde over a Teflon J-wire. The catheters were inserted percutaneously and passed without fluoroscopic control. No obvious complications occurred. The position of both catheters was checked by routine supine A-P chest x-ray. The zero pressure level was defined as mid-left atrium by ECHO measurements. Thermal dilution cardiac output (CO) was obtained using an IL or Edwards CO computer. Iced

injections (10 cc) were used at a standard p.s.i. and flow rate. Five separate CO measurements were performed at each time period, and the average of these was used.

Studies

Serial ECHO studies were performed in the following clinical situations:

1. Postburn resuscitation (0-48 hr).
2. Persistent burn shock.
3. Acute respiratory failure.
 - A. Early respiratory failure (< 60 hours postburn).
 - B. Late respiratory failure (> 5 days postburn).
 - C. Treatment with continuous positive pressure ventilation (CPPV).
4. Septic shock.

Statistical analysis included the Student's t -test and one-way analysis of variance. Significance was defined as a $p < 0.05$. Patients were grouped or matched depending upon the type of clinical category involved.

RESULTS

Complete pressure-volume relationships were measured in 13 patients. A total of 93 individual measurements were analyzed. Echocardiography was performed in all patients, thermal dilution-cardiac output (TD-CO) was measured in 11/13, and right- and left-sided pressures together were available in 9/13. Left ventricular catheters were used in 4/13, all with right-sided pressures and TD-CO. Serial measurements were available in all 13 cases (range 3-14).

Table 3 lists the correlations of LV end-diastolic volume (EDV) to pulmonary artery wedge (PAW), TD-CO, ejection fraction (EF) and mean velocity of diameter shortening (V_{cf}). The ECHO measured LV-EDV was linearly related to PAW.

The correlation between ECHO-CO and TD-CO is more difficult to evaluate. For the entire population, the correlation was linear, with an r of .97. However, when the cardiac outputs are compared at different study phases, some discrepancies occur. In the resuscitation study, the overall r was .79, but differed considerably in the 12- to 24-hour period. Here the r was only .50 in the colloid group. Those treated with crystalloid had a decreased correlation in the

Table 3. Correlation of Echocardiographic Measurements to Invasive Monitoring

Measurement	r
EDV versus PAW	.76
EDV versus EF	.30
EDV versus CO	.70
ECHO-CO versus TD-CO	.97
EF versus PAW	.03

Abbreviations same as Table 1; r = coefficient correlation.

0- to 12-hour period ($r = .69$). In the 24- to 48-hour period, the correlation was excellent.

The ECHO-CO tended to underestimate the TD-CO by 1.8 liters per minute. This was a systematic error and not random. Using plus or minus 15%, the ECHO method underestimated CO in 31% of the individual studies, overestimated in 29%, and was within $\pm 15\%$ in 40% of the individual studies. This was not consistent in any one protocol nor serially in individuals but tended to change with time and/or therapy. Those patients studied with acute respiratory failure treated with CPPV showed the best individual correlation (overestimation 13%, underestimation 25%, with 63% the same).

DISCUSSION

Echocardiography is a well-established diagnostic tool in cardiovascular disease. The technical difficulties are well worked out and include segmental contraction abnormalities, respiratory changes, transducer position difficulties, thoracic cage diameter, reproducibility and technical difficulties with ultrasound reflections (19-21). The utilization of serial ECHO to evaluate the LV function of critical care patients has been a neglected area of study. This report is the final analysis of the ECHO data obtained in thermally injured patients and their postburn complications. It establishes this technique as a useful adjunct or alternative to invasive monitoring in the critically ill patient.

Quantitative data could be obtained in approximately 85% of the patients studied. In another 10%, some qualitative estimate of LV function and volume was possible. In approximately 5% of the patients, no useable echograms could be obtained. Occasionally, a patient could be studied in the early postburn period but could not be echoed at a later date. The reasons for this were varied but

included anterior thoracic wall surgery, respiratory failure requiring mechanical ventilation, pneumothorax, marked respiratory fluctuations, and marked chest wall edema. However, reproducibility of ECHO quality in most patients (96%) was excellent, regardless of intervening clinical complications.

The study emphasis over the last two years has been on three distinct clinical areas of greatest importance in a large burn center: (1) postburn fluid resuscitation, (2) respiratory failure (RF), and (3) sepsis. The data presented offer a better understanding of hemodynamic profiles and LV function during postburn shock, during the treatment of RF with CPPV, early and late RF, septic shock, and dopamine therapy.

The inability to maintain an adequate circulation, based on arterial pressure and CO, has often been ascribed to LV failure. The etiology of this decreased performance has often been attributed to a serum myocardial depressant factor. This speculation is not based on any direct measurements of LV function in man. The measured ECHO LV ejection indices in this study do not support this theory in the case of postburn shock, hyperdynamic septic shock, nor with the treatment of RF with CPPV. Autopsy data correlates well with the findings in this study. In postburn shock and CPPV therapy, the primary finding was a decreased LV intravascular volume. The only substantial evidence of myocardial depression was in two patients with myocardial abscess septic shock.

An important consideration in critical care echocardiography is how various measurements correlate with established invasive techniques. In this respect, the LV ECHO-EDV was linearly related to the PAW and TD-CO. Therefore, it accurately reflected indirectly measured LV filling pressure and CO changes. This was true at low, normal and high levels. Echocardiographic CO compared to TD-CO was more difficult to analyze but was similar to other types of comparison of CO methods.

The ECHO LV ejection indices combined with the pressure-volume measurements have defined a more precise categorization of cardiopulmonary change in the early postburn period. Various therapeutic modifications are suggested by these studies. In the immediate postburn period (first 24 hours), the patient presents with an obligatory fluid loss which requires aggressive volume replacement. The composition, rate of infusion, and total volume of resuscitation fluid are dependent on the percentage of total body surface area involved and the presence or absence of inhalation injury. The early hemodynamic profile, regardless of fluid composition (crystalloid alone versus a colloid-crystalloid combination) is a decreased pressure-volume situation with excellent LV ejection dynamics. This occurred in both crystalloid- and colloid-treated groups. A small percentage (2-4%) of

large burns will exhibit "persistent burn shock" which is manifested by persistent decreased CO, irreversible metabolic acidosis, and hypercontractile LV performance despite massive volume replacement. All of these patients had severe inhalation injury requiring intubation following acute RF. They died within 60 hours postburn, after temporary improvement with colloid therapy. No evidence of myocardial depression was noted, and inotropic therapy was not indicated. These patients must be recognized early, require close invasive monitoring, intubation and volume ventilation, and perhaps colloid therapy. Even with this approach, their prognosis is grave.

In those patients during postburn resuscitation who are not classified as "persistent burn shock," the second 12-hour period appears crucial in volume therapy. During this period, those treated only with crystalloid volume replacement failed to achieve a normal CO. This was primarily because of a persistent decrease in the LV intravascular volume. The LV ejection dynamics remain excellent and not different from the colloid-treated group.

In the second 12 hours, those patients with decreased CO and/or early RF should receive colloid therapy if the primary aim is to re-establish normal hemodynamics, especially in individuals with > 40% total body surface burn. In both groups of patients, there was no evidence of myocardial depression, and therefore no basis for inotropic therapy in the first 24 hours. The urine output and systolic arterial pressure were not useful guides to the adequacy of CO restoration. Once treated with colloid in the second 24 hours, those patients with low CO returned to normal and did not differ from colloid-crystalloid treated patients.

Respiratory failure in the early postburn period is an infrequent occurrence. It usually occurs at < 60 hours postburn and is not distinguished by hemodynamic profiles. However, it has a high mortality (91%) and is often associated with inhalation injury (55%). The type of resuscitation (45% colloid-crystalloid, 55% crystalloid) was not different. All had a large percentage of facial burns (4.8 ± 2.3 percent). Alveolar arterial gradients on room air are generally abnormal (67%). The exact etiology of this syndrome of early RF is unknown, but it is suspected that pulmonary capillary permeability is altered either secondary to the burn or pulmonary injury. All of those with early RF exhibited excellent LV hemodynamic profiles.

Therefore, the ECHO measurements have established three distinct hemodynamic subgroups:

- (1) Those individuals with postburn circulatory compromise, no early respiratory insufficiency. The institution of early colloid therapy (12-24 hours) in this group more rapidly and efficiently re-establishes CO and LV-EDV.

- (2) Thermally injured patients with postburn circulatory compromise, and immediate acute RF.
- (3) Those individuals with "persistent burn shock."

Further controlled studies with alternate therapeutic modalities are necessary to increase the survival rate in the latter two categories. Their rapidly fatal clinical course is not related to LV failure.

The two major complications that occur after the initial 40-60 hours postburn are sepsis and respiratory failure. Those patients who develop late RF usually do so at 6 ± 2 days. This coincides with the time of sepsis in most cases. There are exceptions, but the majority (85%) fall into that time frame. These patients differ from those with early RF in that 86% have abnormal CXRs prior to or at the time of failure. Seventy-one percent have positive blood cultures, while only a small percentage (36%) have an absolute diagnosis of inhalation injury, and their mortality is less (71% versus 91% early RF). Approximately 30% had received Sulfamylon therapy prior to RF. Eighty-two percent had abnormal A-A gradients on initial evaluation (67% in early RF). The etiology was primarily sepsis. Two appeared to have postoperative volume overload, and one had an acute cardiomyopathy.

Echocardiographic LV hemodynamic profiles were useful in establishing the presence of cardiovascular abnormalities in late RF. The patients could be divided into three categories: (1) those with normal LV ejection dynamics with presumed pulmonary failure, (2) those with mildly abnormal LV ejection hemodynamics and mixed cardiopulmonary difficulties, and (3) those with obvious LV failure without a pre-existing pulmonary component. The latter two categories required invasive monitoring.

Echocardiographic measurements during CPPV treatment of late RF revealed that the decreased CO often seen is primarily due to a decreased LV-EDV and not myocardial depression. Therefore, the treatment should be aimed at increasing intravascular volume and maintaining an adequate PaO_2 with the lowest CPPV level.

The other major hemodynamic complication postburn is septic shock. Utilizing the ECHO technique, septic shock was divided into hyperdynamic (HSS), low output (LOSS), and myocardial abscess (MASS). Dopamine therapy was temporarily useful in LOSS but of little value in HSS or MASS. Myocardial depression was not found in HSS nor in two patients with LOSS. Myocardial abscess septic shock was obviously secondary to direct myocardial involvement.

In summary, the ECHO measured LV ejection dynamics and volume correlated well with indirect invasive estimates of cardiovascular function. Echocardiography can be serially performed in critically

ill patients and offers a more precise definition of postburn hemodynamics, including those associated with major complications. In addition, no evidence of an active myocardial depressant factor was recognized in postburn clinical entities in which it had been previously described.

PRESENTATIONS

None

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*		2. DATE OF SUMMARY*		REPORT CONTROL SYMBOL DD FORM 1498 (AR) 636	
3. DATE PREV. SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISSEM INSTR*	8B. SPECIFIC DATA - CONTRACTOR ACCESS		9. LEVEL OF SUM	
77 10 01	D. CHANGE	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO		A. WORK UNIT	
10. NO. CODES*		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY		61101A		3A161101A91C		00		090	
B. CONTRIBUTING									
C. CONTRIBUTING									
11. TITLE (Precede with Security Classification Code)* (U) Measurement of Pulmonary Tissue Volume in Thermally Injured Soldiers (44)									
12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 003500 Clinical Medicine									
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY			16. PERFORMANCE METHOD		
77 04		Cont		DA			C. In-House		
17. CONTRACT GRANT Not Applicable				18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS		B. FUNDS (In thousands)	
A. DATES/EFFECTIVE		EXPIRATION:		FISCAL YEAR		78		.5	
B. NUMBER*		C. TYPE		D. AMOUNT:		79		.6	
E. KIND OF AWARD:		F. CUM. AMT.						13	
								25	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION					
NAME: US Army Institute of Surgical Research				NAME: US Army Institute of Surgical Research					
ADDRESS: Ft Sam Houston, Texas 78234				ADDRESS: Ft Sam Houston, Texas 78234					
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)					
NAME: Basil A. Pruitt, Jr, COL, MC				NAME: Victor Lam, MAJ, MC					
TELEPHONE: 512-221-2720				TELEPHONE: 512-221-6532					
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22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burn injury; (U) Pulmonary function tests; (U) Plasma oncotic pressure; (U) Pulmonary extravascular water; (U) Resuscitation; (U) Humans									
23. TECHNICAL OBJECTIVE* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)									
23. (U) To evaluate the significance of pulmonary extravascular lung water changes in burned soldiers and assess the effectiveness of conventional therapy.									
24. (U) With the disappearance of an indicator soluble gas during a rebreathing maneuver, pulmonary extravascular water will be determined and correlated with changes in arterial blood gas tension and body weight.									
25. (U) 7710 - 7809 Serial pulmonary extravascular water measurements have been completed for twelve patients. Six subjects have been studied under a Ringer's lactate versus Ringer's lactate plus salt-poor albumin resuscitation protocol. Data regarding weight, burn size, fluid intake, urine output, arterial-alveolar oxygen tension gradient and plasma oncotic pressure will be compared with lung water. Pulmonary extravascular water appears to increase steadily for the first 72 hours post-thermal injury.									

*Available to contractors upon originator's approval.

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PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68
AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MEASUREMENT OF PULMONARY TISSUE VOLUME IN THERMALLY
INJURED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

Victor Lam, M.D., Major, MC
Cleon W. Goodwin, Jr., M.D., Major, MC
Richard C. Treat, M.D., Major, MC
Dianne L. Martin, SP5

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

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The rebreathing method for determination of pulmonary extra-vascular water was utilized to monitor lung water changes during the early postburn period. The data from 10 patients show a mean cardiac output of 7.9 liters per minute, lung tissue volume of .851 liters, oncotic pressure of 24.5 torr, age of 32.3 years, and 42.8% total body surface area burn.

Hemodynamic measurements suggest that increased vascular permeability may be the major factor in the development of adult respiratory distress syndrome. No definite conclusions can be reached without further measurements.

Pulmonary extravascular water
Adult respiratory distress syndrome
Pulmonary capillary blood flow
Pulmonary capillary permeability
Plasma oncotic pressure
Resuscitation

MEASUREMENT OF PULMONARY TISSUE VOLUME IN THERMALLY INJURED SOLDIERS

Monitoring the alterations in pulmonary extravascular water volume in acute thermal injury and postburn resuscitation is difficult technically and usually requires invasive techniques (1). The re-breathing maneuver with measurement of the disappearance of dimethyl ether (DME) to determine lung tissue volume has been employed (2,3). This noninvasive test allows serial lung water studies without the risks of infection and bleeding associated with Swan-Ganz and left ventricular catheters.

The potential areas for research with lung tissue volume include the effect of fluid resuscitation composition, various therapeutic modalities, and the diagnosis of early pulmonary edema (adult respiratory distress syndrome). Postmortem lung weights of dogs have shown a good correlation between pulmonary tissue volume and wet lung weights measured by gravimetric methods (2). Clinical application of pulmonary rebreathing measurements includes identification of inhalation injury, early prevention of adult respiratory distress syndrome, and evaluation of cardiac output.

This study evaluated pulmonary extravascular tissue volume during the first 72 hours post-thermal injury in a group of patients receiving the Baxter-crystalloid resuscitation and a group receiving the Brooke colloid formula.

Correlation of pulmonary extravascular water was made with:

- 1) CXR findings.
- 2) Body weight.
- 3) Plasma oncotic pressures.
- 4) Alveolar-arterial O_2 gradients.
- 5) Fluid balance records.
- 6) Clinical status.

1. Morgan A, Knight D, O'Connor N: Lung water changes after thermal burns. *Ann Surg* 187:288-293, 1978.

2. Peterson BT, Petrini MF, Hyde RW, Schreiner BF: Pulmonary tissue volume in dogs during pulmonary edema. *J Appl Physiol: Respirat Environ Exercise Physiol* 44:782-794, 1978.

3. Lam V, Petrini M, Peterson BT, Dale RC, Hyde RW: Effect of continuous positive airway pressure (CPAP) on pulmonary tissue volume (V_t) and pulmonary capillary blood flow (Q) in normal humans. *Circulation* 56 (Suppl III):75, 1977 (Abstract).

TECHNIQUE

All studies were performed in the Institute of Surgical Research Pulmonary Function Laboratory with a modified Perkin-Elmer medical mass spectrometer. Modifications to the mass spectrometer included a heated stainless steel capillary inlet, DME plate at mass 15, and Swagelok inlet connector.

A bag-in-box with a 16" pillow rebreathing bag (Calibrated Instruments) was connected via large bore tubing to an Ohio 843 data acquisition dry spirometer for a volume signal output. A four-way Hans-Rudolph pulmonary breathing valve was used as a mouthpiece and to select between room air and the rebreathing bag. A Honeywell 1858 fiberoptic recorder with a frequency response of 5,000 Hertz was used to record the signal levels of helium, DME, and bag volume.

PROCEDURE

The initial bag volume was adjusted with a test gas mixture of 1.5% DME, 7% helium, 30% oxygen, and balance nitrogen to approximate the forced expiratory volume in one second. Bag concentration of test gas was measured prior to the rebreathing maneuver, and a volume calibration was obtained.

The subject with nose clip was asked to breathe quietly through the mouthpiece and then instructed to empty lungs to residual volume. Then the valve selected the rebreathing bag, and a maximal rebreathing maneuver began for five breaths. Finally, the mixed expired bag gas concentrations were determined.

COMPUTATIONS

The signal trace recording was entered via a digitizer attachment to the Hewlett-Packard minicomputer. Entry of calibration data for volume, time, and initial gas concentration was stored by the BASIC program. Correction of time for passage of gas through the anatomic dead space was performed manually. The first end-expiratory point was corrected in value for anatomic and apparatus dead space.

Calculated values included lung tissue volume, (V_t , Equation 2), residual lung volume (RV), alveolar lung volume (V_A , Equation 4), and inspired lung volume corrected to body temperature pressure saturated (BTPS).

Pulmonary capillary blood flow (\dot{Q}_C , Equation 3) was determined from the slope of a logarithmic normal regression plot. A correlation coefficient R was determined from the goodness of fit of end

EQUATIONS

Initial volume of test gas = volume in alveolar space + volume dissolved in tissue.

$$(1) V_A (100\%) = V_A (\text{intercept } \%) + V_t \alpha_t (\text{intercept } \%)$$

$$(2) V_t = \frac{V_A}{\alpha_t} \left[\frac{100}{\text{intercept}} - 1 \right] \frac{760}{P_B - 47}$$

V_t = pulmonary tissue volume

V_A = alveolar volume

P_B = barometric pressure

α_t = solubility coefficient in lung tissue

Slope of Disappearance Curve

$$(3) \dot{Q}_C = \frac{V_A \frac{760}{P_B - 47} + \alpha_t V_t}{\alpha_b (t_1 - t_2)} \ln \frac{F_A(t_2)}{F_A(t_1)}$$

F_A = concentration of soluble gas in alveoli

\dot{Q}_C = pulmonary capillary blood flow dilution of inert gas

α_b = solubility coefficient in blood

$$(4) V_A = V_I \frac{(F_I)}{(F_E)}$$

V_I = volume inspired

F_I = inspired concentration of insoluble gas (initial)

F_E = expired concentration of insoluble gas (mixed)

tidal points. Rebreathing dead space was obtained from the helium equilibration trace (4).

The validity of individual lung water measurements was analyzed by several criteria. Poor fit of data to a linear regression ($R^2 < 0.98$) was rejected, as were incomplete emptying of rebreathing bag, large residual volumes, poor equilibration of helium trace and evidence of recirculation prior to four breaths.

THEORY

The composition of the lung normally includes 80% water by weight. Measurement of pulmonary tissue volume is therefore a good indication of pulmonary extravascular water space.

Tissue volume measurement via airways with soluble gas disappearance has been shown to be an accurate method for the determination of lung water in pulmonary edema. The vascular method of double indicator dilution requires access both to the right heart (i.e., Swan-Ganz) and to the arterial sampling site and involves a moderate loss of blood during repeat measurements. Thus, it is an invasive procedure that may cause complications and may not be indicated in the mildly ill patient.

The theoretical basis and original breath-holding technique have been reported by Cander and Forster (5). When a soluble gas is inspired into the airways, there will be an equilibrium between the alveolar gas and lung parenchyma (V_t) in about 10 milliseconds (see Figure 1).

The initial drop in soluble gas concentration is a direct function of both the solubility of the gas in lung tissue and the volume of lung tissue. Then in the following 15 seconds, the concentration of soluble gas in the alveoli will decrease exponentially as it equilibrates with and is transported away by pulmonary capillary blood flow. After 15 seconds, there will be an effect from recirculation.

By obtaining serial alveolar gas samples, a disappearance curve can be produced. If plotted as a semilogarithmic graph, the intercept and slope can be determined. The choice of a suitable soluble test gas is dependent on its solubility characteristics. If the gas

4. Petrini MF, Peterson BT, Hyde RW: Lung tissue volume and blood flow by rebreathing: Theory. *J Appl Physiol: Respirat Environ Exercise Physiol* 44:795-802, 1978.

5. Cander L, Forster RE: Determination of pulmonary parenchymal tissue volume and pulmonary capillary blood flow in man. *J Appl Physiol* 14:541-551, 1959.

LUNG MODEL - SOLUBLE GAS

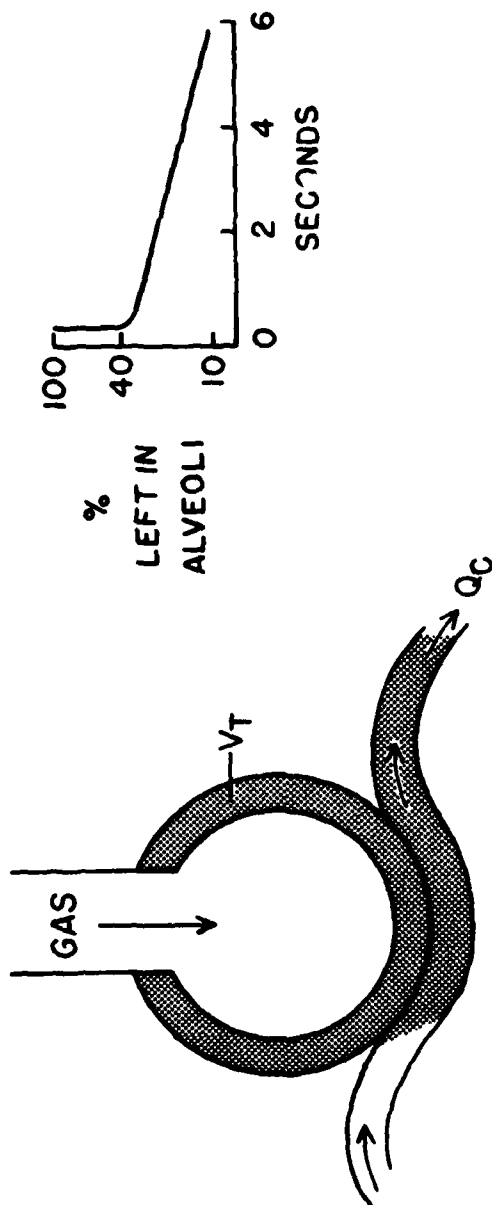


Figure 1

has too high a solubility, then the gas will dissolve into the airway before arriving at the alveoli.

A low solubility gas such as acetylene will produce a small depression in the intercept, and the calculated V_t will be prone to more scatter because of measurement error. Dimethyl ether has been shown to give more precise measurements of V_t with its solubility characteristics $\alpha_t = 9.0$. However, acetylene has been better as an indicator for pulmonary capillary blood flow.

RESULTS

To date, approximately 20 thermally injured patients and 10 normal individuals have had serial pulmonary extravascular water (V_t) determinations. Data for only the first 72 hours postburn have been analyzed. This includes data from 10 patients with a mean age of 32 years, mean percent of burn 43%, mean cardiac output 7.9 liters per minute, V_t .851 liters, and no inhalation injury. Resuscitation fluid composition was not considered in the analysis.

Figure 2 shows the time course of lung tissue volume, with a mean standard error of mean (SEM) depicted. There is a slight progressive increase for the first 60 hours, but the change is not statistically significant. Pulmonary capillary blood flow is shown in Figure 3. Oncotic pressures for five patients are shown in Table 1.

DISCUSSION

Recently, Morgan et al (1) have reported lung water determinations on nine thermally injured patients estimated by lung thermal volumes. There was no statistical analysis of mean lung water volume changes with time. The authors state that two peaks occur in the lung water during early postburn periods prior to maximum peripheral edema and during edema mobilization. It is difficult to evaluate these claims with the data presented.

The time course of pulmonary capillary blood flow does not show any statistically significant changes. We may expect this result when the cardiac output has not been normalized to the individual age of the patient (6). It is difficult to relate changes in cardiac index to normals in the burn literature, since cardiac index has no physiologic basis (6).

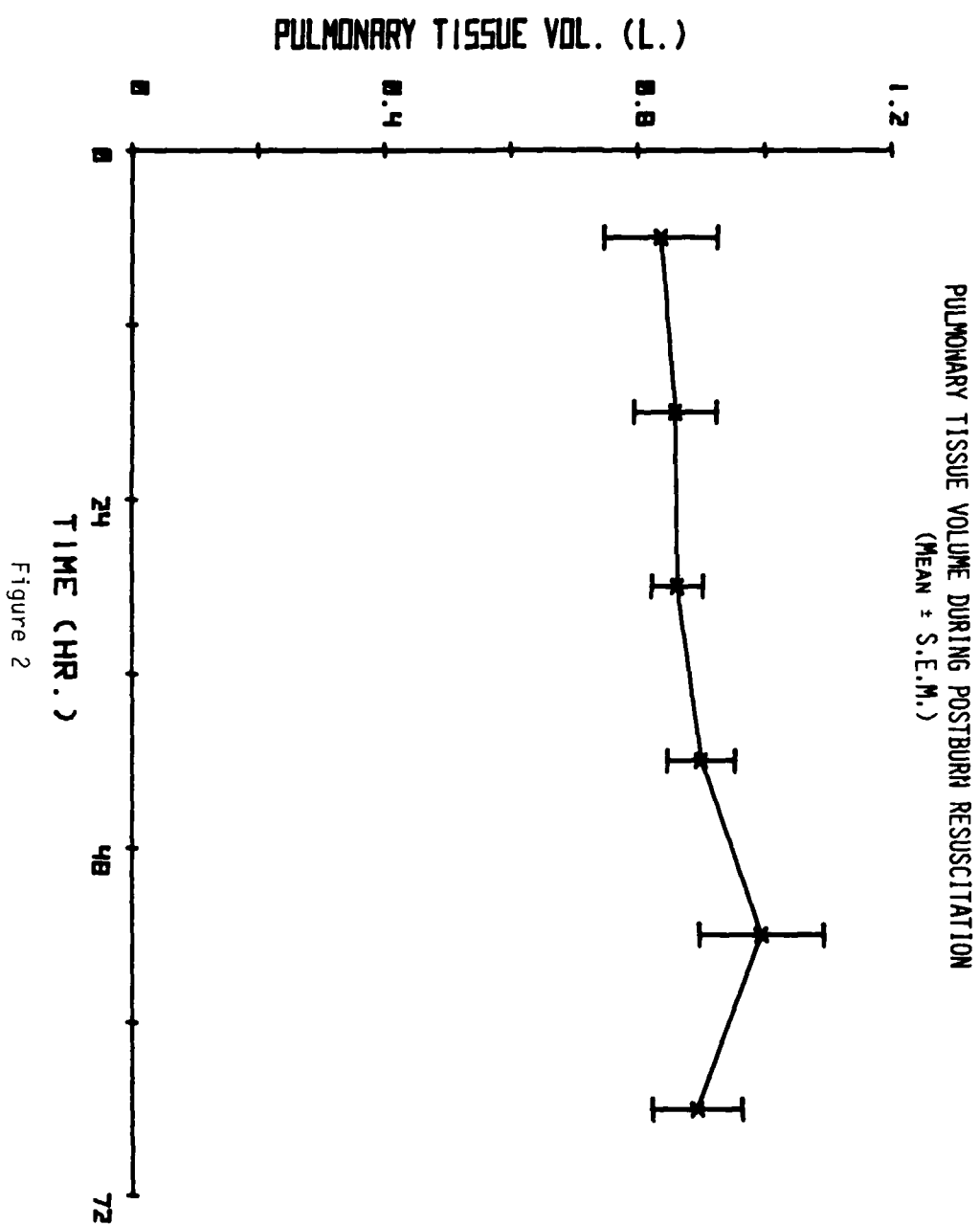
1. Morgan A, Knight D, O'Connor N: Lung water changes after thermal burns. *Ann Surg* 187:288-293, 1978.

6. Guyton AC, Jones CE, Coleman TG: *Circulatory Physiology I: Cardiac Output and Its Regulation*. 2nd edition, Philadelphia, W.B. Saunders Company, 1973.

Table 1

Subject	Age (yr)	% Burn	0-12 hr	12-24 hr	24-36 hr	36-48 hr	48-60 hr	60-72 hr
G.D.	39	37	Onc	17.8	17.8	21.2	24.8	25.4
			CO	4.461	4.865	6.558	6.433	11.251
			Vt	.748	.757	.773	.803	.936
R.G.	40	41	Onc	25.4	23.8	27.2	25.6	28.2
			CO	3.948	5.748	5.924	7.748	6.863
			Vt	.696	.840	.812	1.024	.823
J.C.	24	20	Onc	25.6	26.6	26.2	26.6	26.0
			CO	7.056	7.709	6.467	8.032	9.029
			Vt	1.085	.953	.871	1.115	1.10
J.H.	23	60	Onc	18.1	20.8	19.4	21.8	22.8
			CO	7.12	11.904	9.713	11.38	11.408
			Vt	.856	1.008	.945	.940	.941
R.F.	21	20	Onc	29.4	27.4	28.2	28.8	23.6
			CO	10.955	10.048	10.554	11.07	9.318
			Vt	.913	.761	.727	.663	.589

Onc = oncotic pressure (torr); CO = cardiac output (L/min); Vt = pulmonary tissue volume (liters)



PULMONARY CAPILLARY BLOOD FLOW DURING POSTBURN RESUSCITATION
(MEAN : S.E.M.)

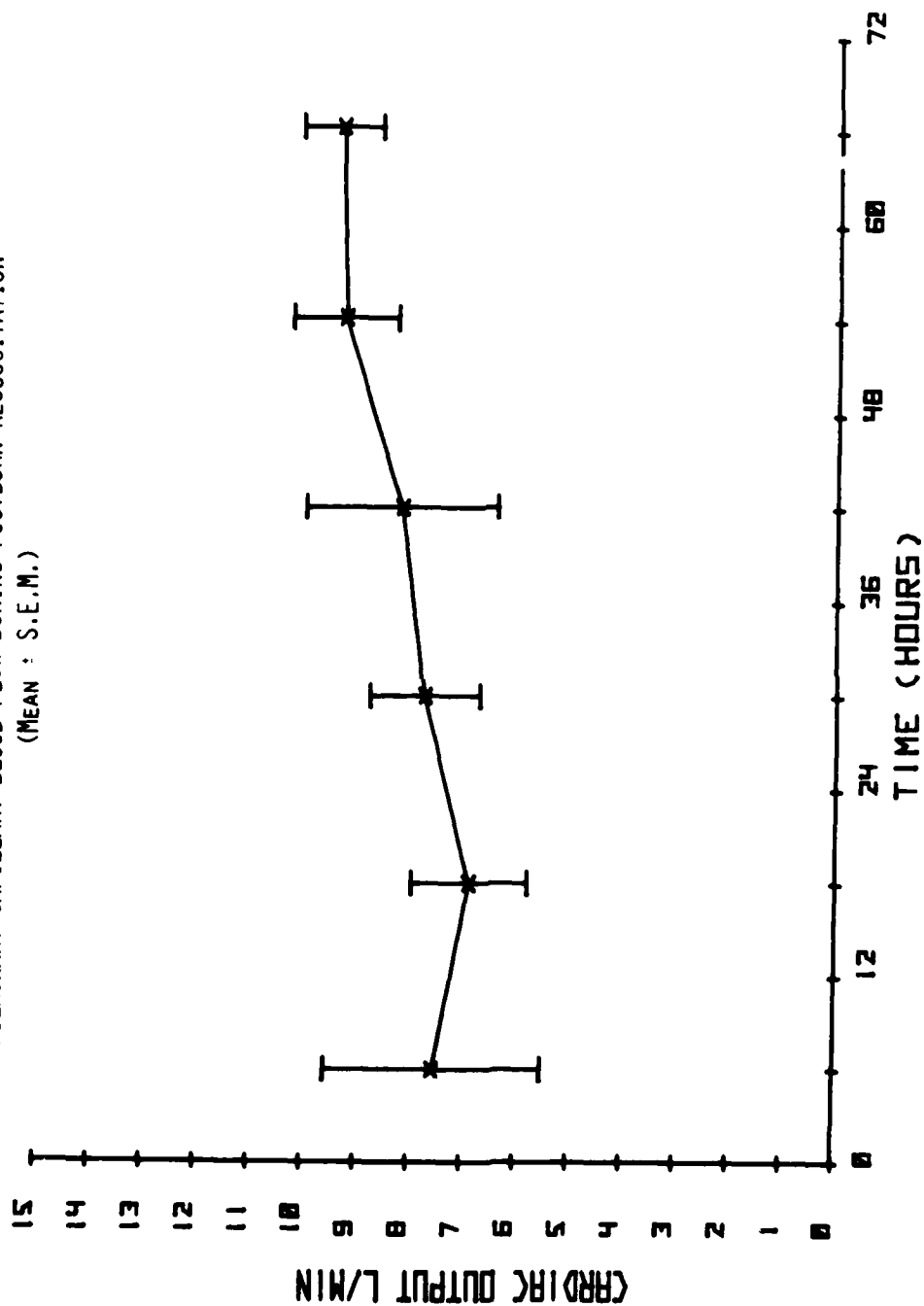


Figure 3

Pulmonary extravascular water by rebreathing technique shows a trend toward higher values during the first 72 hours. The changes in lung tissue volume are not significant by statistical analysis. Parameters such as fluid composition, fluid balance, and body weight will have to be accounted for in the statistical analysis.

The mechanism for adult respiratory distress syndrome appears to be due to a change in vascular permeability rather than microvascular pressure gradients. Generally, pulmonary artery wedge pressures are low to normal, indicating that hydrostatic pressure is not the most important factor in changes of lung fluid balance (J.F. Dorethy, personal communication).

If vascular permeability is the major factor in lung fluid changes in the thermally injured patient, then plasma oncotic pressures may be expected to parallel fluid transfer. Generally, the plasma oncotic pressures in the five patients listed in Table 1 show little change with time. These data do not substantiate the conjecture of Morgan et al (1) regarding the changes in oncotic pressures.

More patients will have to be measured for lung water, and these values need to be correlated to clinically available parameters before definite conclusions can be reached. The rebreathing maneuver for lung water is practical and safe for patient testing. The lung thermal volume method involves a Swan-Ganz pulmonary artery catheter and has resulted in an inordinate risk of bacterial endocarditis in long-term studies (1). Presently, patients are being studied under a resuscitation protocol to evaluate whether a difference in lung water exists for a crystalloid only versus a colloid-crystalloid resuscitation.

1. Morgan A, Knight D, O'Connor N: Lung water changes after thermal burns. *Ann Surg* 187:288-293, 1978.

PRESENTATIONS AND/OR PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY A. CESSION ^a		2. DATE OF SUMMARY ^a		REPORT CONTROL SYMBOL	
				DA OG 6952		78 10 01		DD FORM 1498A, 1 NOV 68	
3. DATE PREVIOUS SUMMARY		4. KIND OF SUMMARY		5. SUMMARY SECURITY		6. SUMMARY SECURITY		7. SUMMARY SECURITY	
77 10 01		D. CHANGE		U		U		NA	
8. NO. CODES ^a		9. PROGRAM ELEMENT		10. PROJECT NUMBER		11. TASK AREA NUMBER		12. WORK UNIT NUMBER	
A. PRIMARY		61101A		3A161101A91C		00		082	
B. CONTRIBUTING									
C. CONTRIBUTING									
13. TITLE (Provide with Security Classification Code) ^a (U) Laboratory Investigation of The Mechanisms of Acquired Leukocyte Dysfunction Following Thermal Injury in Burned Soldiers (44)									
14. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 003500 Clinical Medicine									
15. START DATE			16. ESTIMATED COMPLETION DATE			17. FUNDING AGENCY		18. PERFORMANCE METHOD	
75 12			Cont			DA		C. In-House	
19. CONTRACT GRANT Not Applicable									
A. DATES/EFFECTIVE				B. EXPIRATION				C. FUNDING ESTIMATE	
B. NUMBER ^a				C. TYPE				D. AMOUNT	
A. KIND OF AWARD				F. CUM. AMT.				G. FUNDING ESTIMATE	
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								78	
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								27	
								35	
20. RESPONSIBLE DOD ORGANIZATION					21. PERFORMING ORGANIZATION				
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22. GENERAL USE					23. ASSOCIATE INVESTIGATORS				
FOREIGN INTELLIGENCE NOT CONSIDERED					NAME ^a Arthur D. Mason, Jr., MD				
					NAME ^a				
24. ABSTRACT (Provide SSAN if U.S. Academic Institution)									
(U) Rat model; (U) Burns; (U) Leukocytes; (U) Glucose oxidation; (U) Latex phagocytosis; (U) Stress hormones; (U) Cyclic nucleotides									
25. TECHNICAL OBJECTIVE ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)									
23. (U) Efforts will be made to establish one or more metabolic basis for acquired leukocyte dysfunction following thermal injury. Establishment of specific nutritional or environmental effects may allow for corrective management.									
24. (U) Initial efforts will be to measure glucose metabolism in normal and burned patients' leukocytes. Purified and washed granulocyte populations will be examined for oxidization of Carbon 14 labeled glucose. Hexose monophosphate shunt and glycolysis activity will be estimated by release of ¹⁴ C ₀₂ from respectively 1- ¹⁴ C glucose. Measurements will be made on resting and latex particle (=0.8 u) stimulated cells. Leukocyte function will be examined in the burned rat. The effect of burn rat serum on leukocyte glucose metabolism and cyclic nucleotide levels will be examined. Establishment of an animal model of burn associated leukocyte dysfunction will allow examination of corrective procedures <u>in vivo</u> .									
25. (U) 7710 - 7809 Methods to isolate rat peripheral neutrophils have been established. Assays of glucose metabolism, phagocytosis and <u>in vivo</u> chemotaxis have been established. Sixty percent burned rats have been found to have significant chemotactic depression. The noted chemotactic depression was true for non-specific irritants and bacterial antigens to which the animals were previously immunized.									

^a Available to contractors upon originator's approval

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT
RESEARCH

REPORT TITLE: LABORATORY INVESTIGATION OF THE MECHANISMS OF ACQUIRED
LEUKOCYTE DYSFUNCTION FOLLOWING THERMAL INJURY IN
BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 October 1977 - 30 September 1978

Investigators:

Albert T. McManus, Jr., Captain, MSC
Arthur D. Mason, Jr, M.D.

Reports Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: LABORATORY INVESTIGATION OF THE MECHANISMS OF ACQUIRED
LEUKOCYTE DYSFUNCTION FOLLOWING THERMAL INJURY IN
BURNED SOLDIERS

US Army Institute of Surgical Research, Brooke Army Medical Center,
Fort Sam Houston, Texas 78234

Period covered in this report: 1 October 1977 - 30 September 1978

Investigators: Albert T. McManus, Jr, Captain, MSC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

In the previous reporting period, we documented a marked depression in glucose oxidation by neutrophils from severely burned man (1). The depression was noted in the resting state and during latex phagocytosis. Both the glycolytic and hexose monophosphate shunt pathways were depressed. We proposed that the cells were developmentally defective or were expressing sequelae of the in vivo plasma environment.

We hypothesized that in light of the extreme hormonal alterations following burn stress (2) and the known granulocyte alterations caused by those hormones (3,4), that previously reported burn associated granulocyte defects could be explained by in vivo inactivation of these cells.

1. McManus AT, Jr, Mason AD, Jr: Laboratory investigations of the mechanisms of acquired leukocyte dysfunction following thermal injury in burned soldiers. USAISR Annual Report, p. 352, FY 1977.

2. Wilmore DW, Long JM, Mason AD Jr, Pruitt BA Jr: Stress in surgical patients as a neurophysiologic reflex response. Surg Gynec Obstet 142:25, 1976.

3. Ignarro LS, Lech SY: Bidirectional regulation of lysosomal enzyme secretion and phagocytosis in human neutrophils by guanosine 3'-5' monophosphate and adenosine 3'-5' monophosphate. Proc Soc Exp Biol Med 151:448, 1976.

4. Smith IM, Kennedy LR et al: Adrenergic mechanisms in infection III. Alpha and Beta receptor blocking agents in treatments. Am J Clin Nutr 30:1285, 1977.

This hypothesis has been our experimental focus. We reported in the previous reporting period that the 60% scalded rat had altered granulocyte mobilization to the intraperitoneal injection of casein. This decreased inflammatory response occurred at times when animals had significantly elevated circulating granulocyte counts (5).

We have further investigated this phenomenon. Rats immunized with two injections of 10^9 heat killed *Ps. aeruginosa* on day 30 and day 10 prior to burning had reduced peritoneal inflammatory responses to the injection of 10^9 killed organisms ten days post burn. We have established that the 60% burned rat is significantly more susceptible when surface infected with *Pseudomonas* than a 20% scalded animal.

Granulocyte kinetics following I.V. epinephrine (1:10,000) have been investigated in normal and 60% burned rats. Burned animals were examined 10 days post injury. The following results were observed: Prior to epinephrine injection, burned animals had significantly elevated absolute neutrophil counts ($p < .01$); post epinephrine injection maximal absolute neutrophil counts showed normal animals to have an average 155% rise, burned animals had a significantly reduced response 33% ($p < .01$); additionally, the post epinephrine response of normal animals was not different from the pre-epinephrine absolute neutrophil counts of burned animals. These data indicate an absence of epinephrine responsive neutrophil population at 10 days post burning. This non-responsiveness has been previously reported with less mature rats (170-200 g) with smaller injury (25-30%) (6). In that report, the lack of an epinephrine response was considered as evidence of an absence of a marginating neutrophil reserve. It was also noted that the total peripheral granulocyte count (circulating plus marginating) was below normal in burned animals. This was not our finding. The total peripheral granulocyte count was greater in burned animals. The increase in circulating cell more than compensated for the decrease in epinephrine responsive cells..

The proposed hormonal basis of these abnormal neutrophil findings is being investigated. We have recently established that 60% burned animals have significantly elevated urinary catecholamine output. Possible adrenergic mechanisms are being investigated.

5. McManus AT: Alteration of host resistance in burned soldiers. USAISR Annual Report p.5, FY 1977.

6. Eurenus K, Brouse O: Granulocyte kinetics after thermal injury. Am J Clin Path 60:337, 1973.

PRESENTATIONS

McManus AT, Taylor RL, Mason AD Jr: Examination of altered host resistance in an animal model of burn trauma. Accepted for presentation at the 15th National Meeting of the Reticuloendothelial Society, 6-9 December 1978, Charleston, South Carolina.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a		2. DATE OF SUMMARY ^b		3. REPORT CONTROL SYMBOL	
				DA OG 6978		78 10 01		DD FORM 1498, 1 MAR 68	
4. DATE PREV. SUMM.	5. KIND OF SUMMARY	6. SUMMARY SCTY.	7. WORK SECURITY	8. REGRADING	9. DISSEM. INSTN.	10. SPECIFIC DATA- CONTRACTOR ACCESS		11. LEVEL OF SUM.	
	A. NEW	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO		A. WORK UNIT	
12. NO. CODES ^c	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER			
A. PRIMARY	61101A	3A161101A91C		00		078			
B. CONTRIBUTING									
C. CONTRIBUTING									
13. TITLE: (Precede with Security Classification Code) ^d (U) Monitoring and Modification of The Metabolic and Physiologic Alterations Associated With Thermal Injury in Burned Soldiers (44)									
14. SCIENTIFIC AND TECHNOLOGICAL AREAS ^e 003500 Clinical Medicine									
15. START DATE			16. ESTIMATED COMPLETION DATE		17. FUNDING AGENCY		18. PERFORMANCE METHOD		
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19. CONTRACT ORANT				20. RESOURCES ESTIMATE					
Not Applicable				PRECEDING					
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E. KIND OF AWARD:				F. CUM. AMT.		78		.8	
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21. RESPONSIBLE DOD ORGANIZATION					22. PERFORMING ORGANIZATION				
NAME: US Army Institute of Surgical Research					NAME: US Army Institute of Surgical Research				
ADDRESS: Fort Sam Houston, Texas 78234					ADDRESS: Fort Sam Houston, Texas 78234				
RESPONSIBLE INDIVIDUAL					PRINCIPAL INVESTIGATOR (Pursue SSAN if U.S. Academic Institution)				
NAME: Basil A. Pruitt, Jr, COL, MC					NAME: Michael C. Powanda, PhD, MAJ, MSC				
TELEPHONE: 512-221-2720					TELEPHONE: 512-221-4106				
23. GENERAL USE					24. ASSOCIATE INVESTIGATORS				
FOREIGN INTELLIGENCE NOT CONSIDERED					NAME: DA				
25. KEYWORDS (Precede EACH with Security Classification Code) ^g (U) Pathogenesis; (U) Evaluation of therapy; (U) Patient profile; (U) Plasma; (U) Urine; (U) Enzymes; (U) Proteins; (U) Metabolites; (U) Rats									
26. TECHNICAL OBJECTIVE, 27. APPROACH, 28. PROGRESS (Pursue individual paragraphs identified by number. Precede text of each with Security Classification Code.)									
<p>23. (U) To develop a profile of plasma/urine constituents which accurately reflect the severity of the thermal trauma, critical organ function and the healing process so as to allow objective assessment of the efficacy of therapeutic measures. To use this profile to further elucidate the pathogenesis of thermal injury by studying selected aspects of host metabolism and the factors which regulate these aspects of metabolism in seriously burned and burned infected soldiers and animal models; and ultimately to lessen morbidity and mortality due to severe thermal trauma as well as to hasten convalescence.</p> <p>24. (U) Animal and clinical studies will be run concomitantly when feasible. Animal studies which can be rigorously controlled, will be used to test hypotheses and to expand upon the findings from patient studies. The rat burn model developed by Walker and Mason, suitably modified, will be the primary animal model employed because it allows the use of statistically significant numbers of animals at a reasonable cost and because histologic microbiologic and metabolic correlations can be carried out in the same animal. Rather than study all of the patients which are hospitalized with burns, initial studies will focus on patients which according to age and burn size are deemed to have a 40-60% chance of survival.</p>									

^a Available to contractors upon originator's approval

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: Monitoring and Modification of the Metabolic and Physiologic Alterations Associated With Thermal Injury

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

1 March 1977 - 30 September 1978

Investigator:

Michael C. Powanda, Ph.D., Major, MSC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MONITORING AND MODIFICATION OF THE METABOLIC AND
PHYSIOLOGIC ALTERATIONS ASSOCIATED WITH THERMAL INJURY

US Army Institute of Surgical Research, Brooke Army Medical Center, Fort
Sam Houston, Texas 78234

Period covered in this report: 1 March 1978 - 30 September 1978

Investigator: Michael C. Powanda, Ph.D., Major, MSC

Reports Control Symbol MEDDH-288(R1)

Upon receipt of a Gilford System 3500 computer directed analyzer and a Hyland laser nephelometer in late May, evaluation of the reliability of the tests associated with each system was begun. In regard to the Gilford Analyzer the following analyses for serum constituents have been appraised: hydroxybutyrate dehydrogenase, phosphohexoisomerase, glutamate-pyruvate transaminase, gamma glutamyl transpeptidase, creatinine phosphokinase, lysozyme, albumin, total protein, creatinine, BUN and phosphate. In respect to the laser nephelometer the following tests have been assessed: IgG, IgM, IgA, albumin, transferrin, C-reactive protein, α_1 -acid glycoprotein, α_1 -antitrypsin, α_2 -macroglobulin and haptoglobin. These tests in conjunction with others already in use in the laboratory or yet to be initiated will be used to monitor the status of burn patients and elucidate the pathogenesis of thermal trauma infection in rats.

Studies on rats receiving a 30% dorsal burn infection with Pseudomonas aeruginosa have just begun thus no data are available as yet. Patient studies await clinician collaboration in the study as well as approval of the protocol by the Human Use Committee.

Pathogenesis
Evaluation of therapy
Patient profile

PUBLICATIONS

1 October 1977 - 30 September 1978

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